

NOTICE TO READER

This Background Review Document contains data, a proposed list of substances, and minimum procedural standards that were reviewed by an independent Expert Panel in May 2002.

The reader is referred to the final report entitled, “ICCVAM Evaluation of *In Vitro* Test Methods For Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays,” (NIH Publication No. 03-4503) for the final ICCVAM recommended substances and minimum procedural standards.

[HTML BRD Page](#)

**Current Status of Test Methods
for Detecting Endocrine Disruptors:
In Vitro Estrogen Receptor
Binding Assays**

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LIST OF ACRONYMS AND ABBREVIATIONS

A	Observed anisotropy
A _o	Anisotropy at 0% inhibition
A ₁₀₀	Anisotropy at 100% inhibition
A _b	Anisotropy values of bound ligand
A _f	Anisotropy values of free ligand
ACC	American Chemistry Council
AR	Androgen receptor
B _{max}	The number of binding sites in a cytosolic preparation
BRD	Background Review Document
CASRN	Chemical Abstracts Service Registry Number
CMA	Chemical Manufacturers Association
cDNA	Complimentary DNA
Ci	Curies
DDT	Dichlorodiphenyltrichloroethane
def	Ligand binding domain of the estrogen receptor protein
DES	Diethylstilbestrol
DMSO	Dimethyl sulfoxide
DPM	Disintegrations per minute
ED	Endocrine disruptor
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDTA	Ethylenediamine tetraacetic acid
EPA	(U.S.) Environmental Protection Agency
ER	Estrogen receptor
ER	Estrogen receptor alpha
ER	Estrogen receptor beta
ER _b	Bound estrogen receptor
ER _f	Free estrogen receptor

ER _t	Total estrogen receptor
ERE	Estrogen response element
F _b	Fraction of ligand bound
FDA	(U.S.) Food and Drug Administration
FES1	Fluormone intrinsically fluorescent non-steroidal estrogen
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FFDCA	Federal Food, Drug, and Cosmetic Act
fmol	Femtomole
FP	Fluorescent polarization
FQPA	Food Quality Protection Act
g	Gravity
GLM	General linear models
GLP	Good Laboratory Practices
GST	Glutathione- <i>S</i> -transferase
GST-aERdef	Glutathione- <i>S</i> -transferase fusion protein containing the ligand binding domain of the lizard (anole) estrogen receptor
GST-cERdef	Glutathione- <i>S</i> -transferase fusion protein containing the ligand binding domain of the chicken estrogen receptor
GST-ERdef	Glutathione- <i>S</i> -transferase fusion protein containing the ligand binding domain of the estrogen receptor
GST-hER def	Glutathione- <i>S</i> -transferase fusion protein containing the ligand binding domain of human estrogen receptor alpha
GST-mER def	Glutathione- <i>S</i> -transferase fusion protein containing the ligand binding domain of mouse estrogen receptor alpha
GST-rtERdef	Glutathione- <i>S</i> -transferase fusion protein containing the ligand binding domain of the rainbow trout estrogen receptor
HAP	Hydroxyapatite
HDT	Highest dose tested
hER	Human estrogen receptor alpha
hER -FP	Human estrogen receptor alpha as measured by FP
hER	Human estrogen receptor beta

HPTE	2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1-trichloroethane
I _%	Percent inhibition
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IC ₅₀	Concentration of test substance (inhibitor) that displaces 50% of the reference estrogen from the receptor in a competitive binding assay
kDa	Kilodaltons
K _d	Dissociation or binding constant
K _i	Equilibrium dissociation constant of a receptor-ligand complex
L _b	Bound ligand
ln	Natural log
L _t	Total ligand
μg	Microgram
μL	Microliter
μM	Micromolar
M	Molar
MCF-7	Cell line derived from a human mammary adenocarcinoma
mL	Milliliters
mM	Millimolar
mmol	Millimoles
MUC	Mouse uterine cytosol
NAS	(U.S.) National Academy of Sciences
NCTR	National Center for Toxicological Research
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
nM	Nanomolar
NSB	Non-specific binding
NTP	(U.S.) National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCB	Polychlorinated biphenyl

pM	Picomolar
pmol	Picomoles
ppm	Parts per million
QA	Quality assurance
QSAR	Quantitative structure activity relationship
r _I	Intra-class correlation
RBA	Relative binding affinity
RBC	Rabbit uterine cytosol
rER	Rat estrogen receptor beta
RUC	Rat uterine cytosol
SAB	Science Advisory Board
SAP	Scientific Advisory Panel
SDWA	Safe Drinking Water Act
SE	Standard error
SERM	Selective Estrogen Receptor Modulator
TEDG	Buffer containing Tris, EDTA, dithiothreitol, and glycerol buffer; used in the isolation of ER from animal tissues
Tris	Tris(hydroxymethyl)aminomethane
TSCA	Toxic Substances Control Act
WWF	World Wildlife Fund

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PREFACE

The Food Quality Protection Act and Amendments to the Safe Drinking Water Act in 1996 directed the U.S. Environmental Protection Agency (U.S. EPA) to develop and validate a screening and testing program, to determine whether certain substances may have hormonal effects in humans. In response, the U.S. EPA developed an Endocrine Disruptor Screening Program (EDSP). The U.S. EPA is currently evaluating the scientific validity of screening and testing methods proposed for incorporation into the EDSP. *In vitro* estrogen receptor (ER) and androgen receptor (AR) assays have been proposed as possible components of the EDSP Tier 1 screening battery. The U.S. EPA asked the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to evaluate the validation status of these *in vitro* assays. ICCVAM, which is charged with coordinating the technical evaluations of new, revised, and alternative test methods, agreed to evaluate the assays based on their potential interagency applicability and public health significance.

In order to assess the current validation status of these *in vitro* methods, it was first necessary to compile all of the available data and information for existing assays. The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), which provides operational support for the ICCVAM, subsequently arranged for preparation of this Background Review Document (BRD) by its support contractor, Integrated Laboratory Systems, Inc. (ILS) with financial support from the U.S. EPA. This BRD reviews available data and procedures for existing *in vitro* ER binding assays and is organized according to published guidelines for submission of test methods to ICCVAM (ICCVAM, 1999). Separate BRDs have also been prepared for *in vitro* AR binding assays, *in vitro* ER transcriptional activation assays, and *in vitro* AR transcriptional activation assays.

As part of the ICCVAM evaluation, the U.S. EPA also asked for development of minimum performance criteria that could be used to define an acceptable *in vitro* ER binding assay. It was envisioned that these criteria would be based on the performance of existing standardized *in vitro* ER binding assays. The minimum performance criteria could be used to assess the acceptability of other new or revised assays proposed in the future. However, a comprehensive review determined that there were no standardized *in vitro* ER binding assays with adequate validation

data that could serve as the basis for establishing these performance criteria. An independent Expert Panel (Panel) will therefore be convened to assess the status of existing *in vitro* ER binding assays and to develop recommendations for standardized assays and validation studies that should be conducted. After adequate validation studies have been completed on one or more standardized ER binding assays, an independent Peer Review Panel will be convened to evaluate the validated assay(s) and to recommend minimum performance criteria for *in vitro* ER binding assays.

This BRD reviews available *in vitro* ER binding assays and presents the data available for substances evaluated in these assays. The relative performance of various types of *in vitro* ER binding assays is compared using this existing data, which was very limited for some of the assays. Based on the comparative performance and advantages and disadvantages of each type of assay, several assays are proposed as priority candidates for standardization and future validation. In addition, minimum procedural standards that should be used for *in vitro* ER binding assays are proposed. These standards include elements such as dose selection criteria, minimum number of replicates, appropriate positive and negative controls, criteria for an acceptable test run, and proficiency standards for participating laboratories. Finally, the BRD proposes a list of substances recommended for the validation of *in vitro* ER binding screening assays.

An Expert Panel was convened in a public meeting on May 21-22, 2002, to review the information and proposals provided in this BRD, and to develop conclusions and recommendations on the following:

- Specific assays that should undergo further evaluation in validation studies, and their relative priority for evaluation.
- The adequacy of proposed minimum procedural standards.
- The adequacy of protocols for specific assays recommended for validation studies.
- The adequacy and appropriateness of substances proposed for validation studies.

The Expert Panel meeting was announced to the public in a *Federal Register* notice (Vol. 67, No. 66, pp. 16415-16416, Apr. 5, 2002; also available on the internet at: <http://iccvam.niehs.nih.gov/docs/FR/6716415.pdf>).

An ICCVAM Endocrine Disruptor Working Group (EDWG) was organized to coordinate the technical evaluation of *in vitro* endocrine disruptor screening methods. The EDWG is co-chaired by Drs. David Hattan and Marilyn Wind, and consist of knowledgeable scientists from ICCVAM agencies. The EDWG functions include identification and recommendation of experts for the Expert and Peer Review Panels, the review of test method BRDs for completeness, preparation of questions for the Expert and Peer Review Panels, and development of draft ICCVAM test recommendations based on Panel evaluations. Final ICCVAM test recommendations are then forwarded from the ICCVAM to Federal agencies for their consideration.

In August 2002, the draft of this BRD was revised to address corrections and omissions noted by the Expert Panel and published as a final version. The final report of the Expert Panel and a proposed list of substances for validation studies of *in vitro* ER and AR methods was published and made available to the public for comment as announced in a *Federal Register* notice (October 2002). A final ICCVAM Test Method Evaluation report will be published in early 2003. This report will include ICCVAM recommendations, the final Expert Panel report, a recommended list of substances for validation studies, and public comments. The report will be forwarded to federal agencies for their consideration and made available to the public.

The efforts of the many individuals who contributed to the preparation, review, and revision of this BRD are gratefully acknowledged. These include Barbara Shane, Christina Inhof, Errol Zeiger, Raymond Tice, Bradley Blackard, Steven Myers, and Linda Litchfield, from ILS, Inc. who prepared the BRD. The suggestions and advice from the ICCVAM EDWG members and co-chairs on early drafts and subsequent versions were invaluable, as were the comments from *ad hoc* reviewers on the final draft. Additional comments and suggestions for improvement of this and future test method documents are welcome at any time.

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EXECUTIVE SUMMARY

The objectives of this BRD are to: (1) provide comprehensive summaries of the published and publicly available unpublished data on the scientific basis and performance of *in vitro* assays used to test substances for their ability to bind to the estrogen receptor (ER); (2) assess the *in vitro* ER binding assays considered for their effectiveness in identifying endocrine-active substances; (3) identify and prioritize *in vitro* ER binding assays that might be considered for incorporation into future testing programs for validation; 4) develop minimum performance criteria by which to judge the effectiveness of proposed *in vitro* ER binding assays; and (5) generate a list of recommended substances to be used in validation efforts.

The data summarized in this BRD are based primarily on information obtained from the peer-reviewed scientific literature. An online literature search was conducted to retrieve records on publications reporting on the testing of substances for their endocrine disrupting effects *in vitro*. Of the 459 records obtained from the initial search, 260 of these citations contained information on ER binding. Data from 72 of these publications were included in this BRD. Some of the peer-reviewed publications that contained ER binding data were not abstracted for inclusion in this BRD because the studies lacked the appropriate details or contained data from unique procedures or substances that were not clearly identified.

Data were abstracted from 14 different ER binding assays. These assays used ER derived from uterine cytosol from the mouse (MUC), rat (RUC), and rabbit (RBC); from MCF-7 cells and MCF-7 cytosol; from human cDNA clones of the two human ER subtypes, ER α and ER β (hER α and hER β), and from cDNA clones of rat ER α (rER α). Fusion proteins in which glutathione-S-transferase (GST) was fused with the *def* domains of the human ER α (GST-hER α), and the ER from mice (GST-mER), chicken (GST-cER), anole (GST-aER), and rainbow trout (GST-rtER) served as the ER source for five assays. All of the assays except one measured the competitive displacement of radiolabeled ($[^3\text{H}]$ or $[^{125}\text{I}]$) 17 β -estradiol from the ER. One assay, designated as hER α -FP, measured the displacement of a fluorescently-labeled estrogen ligand by the test substances using fluorescent polarization (FP).

The majority of the 638 substances tested for *in vitro* ER binding could be classified into one of the following chemical classes: polychlorinated biphenyls, phenolic and non-phenolic steroids, triphenylethylenes, organochlorines, polycyclic aromatic hydrocarbons, stilbenes, phenols, and bisphenols. Only 50% of the substances could be assigned to a product class, the most common of which were pharmaceuticals, pesticides, chemical intermediates, dielectric fluids or their components, natural products (including several phytoestrogens), and plasticizers.

More than half the substances (376; 59%) were tested in the RUC assay, and 133 (21%) of the substances were tested in the next most frequently used assay, hER . For five of the 14 assays (hER -FP, RBC, rER , GST-mER def, GST-cERdef), published data were located on fewer than 50 substances per assay. Only 15 (2.4%) of the substances had been tested in 10 or more assays, and of these, only four (0.6%) had been tested in all 14 assays; in contrast, 403 (63.2%) of substances had been tested in one assay only.

The majority of the publications reported the data as IC₅₀ values or relative binding affinities (RBA), that is, the ratio of the IC₅₀ of the reference estrogen, 17 β -estradiol, divided by the IC₅₀ of the test substance and multiplied by 100.

Although a large number of substances have been tested in these *in vitro* ER binding assays, relatively few have been tested more than once in the same assay or in multiple assays. Furthermore, because the primary focus of many of the studies reviewed in this BRD was on understanding the mechanisms of ER binding, and not on identifying substances with ER-binding activity, much of the published data are of limited value for the analysis of performance or reliability of these assays.

To assess comparative assay performance, a quantitative assessment was conducted using the available IC₅₀ and RBA data after log normal transformation of the data to reduce possible skewness. In this analysis, only positive responses were considered (i.e., discordant positive and negative results for the same substance in the same assay were not taken into account). The quantitative assessment of the data showed that the effect of substances on the variation in RBA and IC₅₀ values was much greater than the effect of assay type, and that there were no significant

differences in performance among the different *in vitro* ER binding assays. This quantitative assessment was limited by the lack of multiple test data within an assay for most of the substances, and by the lack of data across all assays for many substances.

A qualitative assessment of the IC₅₀ data, which considered both negative and positive results, was performed also. This assessment considered whether RBA values (single or median) obtained for substances tested in each of 13 assays were within the same log range as the corresponding values obtained for the same substances when tested in the RUC assay. Based on this qualitative approach, the hER , hER -FP, hER , rER , GST-rtERdef, and MUC assays performed better than the RUC assay; the MCF-7 cytosol assay performed about as well as the RUC assay; and the remaining GST-ERdef assays, the MCF-7 cell assay, and the RBC assay did not perform as well as the RUC assay.

To assess assay reliability, a quantitative assessment was conducted using log normal transformed IC₅₀ and RBA data. Again, only positive responses were considered. An analysis of the variances for the RBA values of 12 substances that had been tested in at least nine of the 14 *in vitro* ER binding assays suggested that there were no statistically significant differences in the reliability of the assays as performed by different laboratories. A comparison of the variability in RBA and IC₅₀ values across assays, ignoring substance effects, suggested that the RUC and hER assays were the most consistent, and that the RBC assay was the least consistent among the 14 assays evaluated. An analysis of the variability in the IC₅₀ for the reference control chemical, 17 β -estradiol, indicated that the most consistent results were obtained with the hER -FP assay, while the MUC, RUC, and hER assays exhibited somewhat greater, but comparable, variances. The low variability associated with the hER -FP assay, however, might be a reflection of the small number of laboratories that have reported IC₅₀ values using this method.

Generally, the databases for all the *in vitro* ER binding assays considered in this BRD are too limited to draw any sound conclusions regarding their performance and reliability. However, based on general principles, recommendations were made in regard to the use of *in vitro* ER binding assays as a component of a Tier 1 endocrine disruptor screening battery:

- Based on a consideration of such factors as relative performance, elimination of animal use, the use of the ER from the species of interest, and the use of alternatives to radioactive substances, the hER , hER -FP, and hER assays should have the highest priority for validation as screening assays for human health-related issues, while the GST-rtERdef assay might be preferred when screening for substances that pose a hazard to wildlife.
- In conducting future validation studies with these assays, the RUC assay should be used as the reference test method. The RUC assay is currently undergoing validation efforts sponsored by the U.S. EPA and the resulting performance and reliability information could be used to establish minimal performance standards for other assays.
- Formal validation studies should be conducted using appropriate substances covering the range of expected RBA values to adequately demonstrate the performance characteristics of the *in vitro* ER binding assays recommended as possible screening assays.
- There is little information about the ER binding activity of metabolites of xenobiotics and it is not clear whether metabolic activation needs to be included in an *in vitro* ER binding test method used as a screening assay. This issue should be considered prior to the implementation of future validation studies.

An important step towards acceptance of an *in vitro* ER binding assay into a regulatory screening program is production of high quality data. To achieve this goal, it is recommended that any future pre-validation and validation studies on *in vitro* ER binding assays be conducted with coded substances and in compliance with GLP guidelines. Ideally, if multiple laboratories are involved in the validation study, the substances should be obtained from a common source and distributed from a central location.

In conducting these validation studies, all of the original data and documentation supporting the validation of a test method must be carefully documented, and include detailed protocols under which the data were produced.

If an assay chosen for validation requires the use of animals, the studies should be conducted to minimize the number of animals used, and animal pain and distress. Adoption of one of the

assays using purified or semi-purified receptors, or glutathione fusion proteins would eliminate the use of animals for *in vitro* ER binding experiments.

Since there are no published guidelines for conducting *in vitro* ER binding studies, and no formal validation studies have been performed to assess the reliability or performance of ER binding assays, the U.S. EPA requested that minimum procedural standards based on a comparative evaluation of *in vitro* ER binding assays be provided. In addition it was requested that a recommended list of test substances be provided for use in validation studies.

The minimum procedural standards include methods for determining the K_d of the reference estrogen, methods for test substance preparation, the concentration range of the test substance (including the limit dose), the use of negative and positive controls, the number of replicates per test substance concentration, dose spacing, assay acceptance criteria, data analysis, evaluation and interpretation of results, minimal information to include in the test report, and the need for replicate studies.

Based on a RUC protocol provided by the U.S. EPA, a suggested general protocol for measuring ER binding using the RUC assay was developed as a potential resource for scientists interested in developing their own laboratory specific protocol. This general RUC protocol incorporated the recommended minimum procedural standards. Various aspects of the assay performance, including preparation of the ER, reagents and solutions, measurement of ER binding, evaluation of the data, and test report guidance are described.

In the development of a list of reference substances for use in validation studies, consideration was given to the number of times the substance had been tested in the RUC assay, the median RBA value of the substance in the RUC assay, and the extent of concordance of the median RBA value in the RUC assay with values obtained for the same substance in other *in vitro* ER binding assays. The substances were then sorted according to their median RBA values, which ranged over seven orders of magnitude. Five substances were selected for each RBA category (≥ 10 , $<10^{-1}$; $<10^{-1}$; <0.01 - 0.1 , <0.01 - 0.001 ; <0.001) and three for a negative category group. Weakly-binding substances (RBA values <0.001) were difficult to identify because they were

not consistently positive in tests within an assay or among different assays. Substances were classified as "negative" for ER binding based on the lack of a positive response in multiple assays when tested at concentrations of at least 1 mM. When possible, representatives of the most common classes of substances were included in each RBA category.

It is anticipated that this BRD and the guidance it provides will help to stimulate validation efforts for *in vitro* ER binding assays.

1.0 INTRODUCTION AND RATIONALE FOR THE USE OF *IN VITRO* ER BINDING ASSAYS

1.1 Introduction

1.1.1 Historical Background of *In Vitro* Endocrine Disruptor Assays and Rationale for Their Development

It is well known that small disturbances in endocrine function, especially during highly sensitive stages of the life cycle (e.g., fetal and prepubertal development), can lead to significant and lasting effects on the exposed organism (Kavlock et al., 1996; U.S. EPA, 1997; NAS, 1999). In recent years, evidence has been accumulating to suggest that exposure to natural and anthropogenic substances in the environment may adversely affect the endocrine and reproductive systems of mammals, fish, reptiles, amphibians, and birds. Substances that cause such effects are classified as “endocrine disruptors”. Disruption of the endocrine system has been demonstrated in laboratory animals and documented in wildlife (Ankley et al., 1998). For example, male fish caught in rivers in many regions of the United States have high levels of vitellogenin, a female-specific protein (Purdom et al., 1994; Folmar et al., 1996), and female mosquitofish living in streams in which pulp mill effluents containing steroidal substances have been discharged possess male gonadal structures (Bortone et al., 1989). The degree to which humans are affected by endocrine disruptors is unknown, although there are reports that these substances might be contributing to increasing incidences of breast, prostate, and testicular cancers (Glass and Hoover, 1990; Adami et al., 1994; Toppari et al., 1996) and to precocious puberty, hypospadias, and decreased sperm counts (Carlsen et al., 1992; Sharpe and Skakkabaek, 1993). However, other investigators have concluded that there is no evidence for endocrine disrupting effects in humans (Safe, 2000; Barlow et al., 1999).

In 1996, the U.S. Congress responded to societal concerns by passing legislation requiring the U.S. EPA to develop a screening and testing program, using appropriately validated test methods, to detect potential endocrine disruptors in pesticide formulations (the Food Quality Protection Act; FQPA) (P.L. 104-170), and in drinking water (the 1996 amendments to the Safe Drinking Water Act; SDWA) (P.L. 104-182). As a result of these mandates, the U.S. EPA formed the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to provide advice on how to best design a screening and testing program for identifying endocrine

disruptors. In August 1998, EDSTAC issued a report recommending that the U. S. EPA evaluate both human and ecological (wildlife) effects; examine effects to estrogen, androgen, and thyroid hormone-related processes; and test both individual substances and common mixtures (U.S. EPA, 1998a). In December 1998, based on these recommendations, the U.S. EPA proposed the EDSP (U.S. EPA, 1998b). In 1999, the EDSP and its proposed approach to screening for endocrine disruptors were endorsed by the U.S. EPA Science Advisory Board (SAB) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP), which also made a number of recommendations concerning the proposed approach (U.S. EPA, 1999).

The EDSP proposes a two-tiered approach for screening and testing. Tier 1 is comprised of *in vitro* and *in vivo* assays and is designed as a screening battery to detect substances capable of interacting with the estrogen, androgen, or thyroid hormone systems. Tier 2 is comprised of *in vivo* assays only and is designed as a testing battery to (1) determine whether an endocrine-active substance (identified in Tier 1 or through other processes) causes adverse effects in animals; (2) identify the adverse effects; and (3) establish a quantitative relationship between the dose and the adverse effect (U.S. EPA, 2000).

The EDSP's proposed Tier 1 screening battery includes the following assays:

In vitro assays:

- ER binding/transcriptional activation (TA) assay
- AR binding/TA assay
- Steroidogenesis assay with minced testis

In vivo assays:

- Rodent 3-day uterotrophic assay (subcutaneous dosing)
- Rodent 20-day pubertal female assay with enhanced thyroid endpoints
- Rodent 5-7 day Hershberger assay
- Frog metamorphosis assay
- Fish gonadal recrudescence assay

The alternative Tier 1 assays include:

- Placental aromatase assay (*in vitro*)
- Modified rodent 3-day uterotrophic assay with intraperitoneal dosing (*in vivo*)
- Rodent 14-day intact adult male assay with thyroid endpoints (*in vivo*)
- Rodent 20-day thyroid/pubertal male assay (*in vivo*)

According to the EDSP, the Tier 1 assays should:

- Detect all known modes of action for the endocrine endpoints of concern;
- Maximize sensitivity to minimize false negatives, while permitting a to-be-determined level of false positives;
- Include a sufficient range of taxonomic groups among the test organisms to reduce the likelihood that important pathways for metabolic activation or detoxification of the test substances are not overlooked; and
- Incorporate sufficient diversity among the endpoints and assays to permit conclusions based on weight-of-evidence considerations.

The proposed Tier 2 testing battery includes the following *in vivo* assays:

- Two-generation mammalian reproductive toxicity assay
- Avian reproduction assay
- Fish reproduction assay
- Amphibian reproduction and developmental toxicity assay
- Invertebrate reproduction

The alternative Tier 2 assays include:

- Alternative mammalian reproductive test
- One-generation mammalian reproduction toxicity test

According to the EDSP, the Tier 2 assays should:

- Encompass critical life stages and processes in mammals (equivalent to humans), fish, and wildlife;

- Encompass a broad range of doses and the administration of the test substance by a relevant route of exposure; and
- Provide a comprehensive profile of biological consequences of substance exposure and relate such results to the causal dose and exposure.

Two proposed *in vitro* components of the Tier 1 screening battery are ER binding/TA assays, and AR binding/TA assays. The primary rationale for inclusion of *in vitro* assays in the EDSP Tier 1 screen is that they:

- Are suitable for large-scale screening;
- Are based on well-elucidated mechanisms of action; and
- Measure specific endpoints.

The Tier 1 assays are informative with regard to the mechanism of action of the presumptive endocrine disruptor and provide guidance for prioritization for further testing. Due to their sensitivity, these *in vitro* tests should permit the identification of an active substance(s) within a complex mixture. TA assays have an advantage over binding assays because they can measure if there is a biological response to receptor binding (i.e., RNA transcription) and thus, unlike binding assays, can distinguish between an agonist (i.e., a substance that mimics the action of endogenous hormones) and an antagonist (a substance that binds to a receptor without eliciting a biological response, blocking the action of endogenous hormones) (U.S. EPA, 1998b). However, it needs to be emphasized that these *in vitro* assays cannot be used to predict the risk for an adverse health effect in humans or wildlife. Binding assays only measure the physical binding of a substance to the receptor while TA assays infer, but do not prove, that an adverse health outcome can occur *in vivo*.

As part of the validation process for the proposed EDSP assays, the U.S. EPA is supporting an effort to prepare a series of BRDs on the Tier 1 *in vitro* ER binding, AR binding, ER TA, and AR TA screening assays. Other EDSP-proposed assays will be evaluated through other organizations (e.g., the U.S. EPA and the Organisation for Economic Co-operation and Development [OECD]). The objectives of each BRD are to:

- Provide a comprehensive summary of the available published and publicly available unpublished data on the scientific basis and performance of the identified assays;
- Identify available assays that might be considered for incorporation into the EDSP;
- Assess their effectiveness for identifying endocrine-active substances;
- Develop minimal procedural standards for acceptable ER and AR binding and TA assays; and
- Provide a list of candidate substances for future validation studies.

1.1.2 Prior or Proposed Peer Reviews of *In Vitro* ER Binding Assays

Although there has been extensive research conducted in the past few years to develop new and improved *in vitro* assays to identify substances with ER binding and transcriptional activity, there have been no formal peer reviews of the validation status of such assays. This BRD has been prepared for an upcoming ICCVAM expert evaluation of the validation status *in vitro* ER binding assays, in concert with reviews of ER TA assays and *in vitro* AR binding and TA assays.

1.2 Scientific Basis for the Proposed Tier 1 *In Vitro* ER Binding Assays

1.2.1 Purpose for Using *In Vitro* ER Binding Assays

In vitro ER binding assays are designed to identify substances (ligands) that bind to the ER and that might act as an estrogenic agonist and cause estrogenic effects, or interfere with normal estrogen activity *in vivo* by acting as an antagonist. The assays can be divided into two mechanistic categories: those that measure binding to the receptor and those that measure transcriptional activation subsequent to binding to the receptor. Although receptor binding assays detect both agonists and antagonists, they do not distinguish between the two. In contrast, TA assays can be designed to distinguish between agonists and antagonists.

Binding of the natural ligand, 17 β -estradiol, to the ER is a prerequisite for the induction of many subsequent estrogenic effects, such as induction of cell proliferation in the uterus, and maintenance of bone and the cardiovascular system. The binding affinity of a xenobiotic substance for the ER determines how well it will compete with 17 β -estradiol. *In vitro* ER competitive binding assays are generally performed by quantifying the ability of substances to compete with 17 β -estradiol for binding. However, ER binding alone is not sufficient to indicate

or predict subsequent cellular effects. For this reason, *in vitro* ER binding assays will be used in conjunction with other *in vitro* and *in vivo* assays for Tier 1 screening. Results from such assays will be used in a weight-of-evidence approach to select substances for Tier 2 testing.

1.2.2 Development of *In Vitro* ER Binding Assays: Historical Background

The foundation of current *in vitro* assays for detecting the ability of substances to bind to the ER can be traced back to the mid-1960s when the receptor was first isolated. At that time, procedures were developed not only for isolation of the receptor but also for the measurement of 17β -estradiol binding and the competitive binding of other substances to the receptor.

The ER was first identified, isolated, and characterized as a protein by Toft and Gorski (1965; 1966) and Noteboom and Gorski (1965) from the soluble fraction of the rat uterus using radiolabeled 17β -estradiol. In these studies, it was shown that the synthetic estrogen diethylstilbestrol competed with 17β -estradiol for binding, but that the non-estrogenic hormones, testosterone and corticosterone, did not bind the ER, and 17β -estradiol was partially inhibitory. Noteboom and Gorski (1965) also initiated the use of radiolabeled (with tritium, [^3H]) 17β -estradiol for measuring receptor binding affinity and showed the response to be stereospecific. These studies were extended by Toft et al. (1967), who showed that a cell-free system derived from rat uterine tissue had the same estrogen-binding properties as were found at physiological concentrations *in vivo*. A Scatchard plot (Scatchard, 1949) was used to determine the dissociation constant of 17β -estradiol for the ER and the number of binding sites in the tissue preparation. The size of the ER was subsequently estimated as 53 kDa. The estimated dissociation constant for 17β -estradiol was 7×10^{-10} M. Notides (1970) demonstrated that the 17β -estradiol dissociation constants for receptors isolated from rat uterus and anterior pituitary were similar (1.55×10^{-9} compared to 1.40×10^{-9} M) and that the responses of these receptors to estrogenic antagonists were essentially identical.

The translocation of the ER complex from the cytosol into the nucleus and its interaction with chromatin was suggested by the work of Shyamala and Gorski (1968) and Jensen et al. (1968). Gorski et al. (1968) hypothesized that the translocated ER complex had DNA-regulatory activity. Clark and Gorski (1969) used a cell-free system to demonstrate that the ER complex bound

equally well to the “nuclear pellet” derived from the uterus, which contains ER, to the kidney, which lacks ER, and to glass pellets. This observation demonstrated that there are no specific nuclear (as opposed to DNA) receptors for the complex.

Between 1965 and 1971, a number of *in vitro* methods were developed to measure the binding of $^{17}\beta$ -estradiol and other substances to the ER. Hähnel (1971) and Jungblut et al. (1972) evaluated a number of these *in vitro* methods using cytoplasmic ERs isolated from calf uteri and human breast cancer tissue. They concluded that the dextran-coated charcoal, Sephadex chromatography, and agar electrophoresis methods for the separation of the receptor-bound ligand from unbound, radiolabeled $^{17}\beta$ -estradiol were suitable for routine use and had equivalent sensitivities. However, Jungblut et al. (1972) concluded that the dextran-charcoal procedure would be the most suitable because its labor, time, and cost requirements were the lowest of the three methods. Hähnel (1971) and Shafie and Brooks (1979) evaluated the effects of other protocol factors on the binding of $^{17}\beta$ -estradiol to the ER and the measurement of unbound fraction. The factors evaluated included pH, storage time of the cytosolic preparation, time and temperature of incubation of $^{17}\beta$ -estradiol with the ER, $^{17}\beta$ -estradiol concentration, sulfhydryl blocking reagents, protein concentration of the cytosol, and the competitive absorption of estrogen to the charcoal. Erdos et al. (1970) developed a hydroxyapatite (HAP)-column binding procedure that was able to distinguish $^{17}\beta$ -estradiol binding to high-affinity versus low-affinity receptor sites.

In the late 1960s and early 1970s, it became apparent that a competitive *in vitro* binding assay would be useful. In such an assay, an ER that contains bound $^{17}\beta$ -estradiol is challenged with other substances to determine if these substances alter its binding. One of the earliest studies was performed by Korenman (1970), who measured the comparative binding affinities of natural and synthetic steroids to rabbit cytosol and compared the results with data from an *in vivo* rodent uterotrophic assay. The correlation between the *in vitro* and *in vivo* responses was considered acceptable. The authors noted that the *in vitro* ER binding assay offered many advantages, but that it could not distinguish between agonists and antagonists.

ER binding assays are most often conducted with a cell-free ER preparation obtained from estrogen-responsive tissues or cells. The current procedures used to isolate ER are essentially the same as those used in the late 1960s and early 1970s. Traditional techniques to measure competitive binding are routinely used as well, including the use of dextran-coated charcoal and HAP to separate receptor-bound ligand from free ligand. Although ER binding assays have changed very little over their 30 plus years of use, some of the newer procedures have incorporated more recently developed technology, including the use of recombinant ER proteins in place of ER isolated from tissues or cells (Matthews and Zacharewski, 2000) and measurement of fluorescence polarization (FP) equilibrium binding in place of the measurement of radioactivity (Bolger et al., 1998). The ER binding assays, as currently performed, are described in detail in **Section 2.0**.

The procedures used to calculate the binding parameters are essentially variations on the method published by Scatchard (1949), who developed models for the binding of small molecules to proteins and for extrapolating binding data. Puca and Bresciani (1968) used Scatchard's procedure to estimate the number of ER binding sites and the 17β -estradiol association constant in isolated calf uterus tissue. In a "Scatchard plot", a straight line indicates that a single class of binding site is present; if competing binding sites are present, the line will deviate from linearity. The intercept on the abscissa indicates the number of binding sites available; the association constant is the ratio of the intercepts on the abscissa and ordinate (Puca and Bresciani, 1968). Scatchard plots are widely used in receptor binding studies.

Baulieu and Raynaud (1970) proposed using an alternative procedure for approximating the binding parameters of small molecules in protein mixtures. They developed a non-linear function by plotting the log of the bound fraction to the log of the total ligand, and demonstrated that this procedure was able to quantitatively distinguish between specific and nonspecific binding in a tissue extract that contained a mixture of specific and nonspecific receptors.

The ER binding assays measure the affinity of radiolabeled 17β -estradiol for the ER (K_d), the affinity of the unlabeled ligand for the ER (K_i), and the concentration at which the unlabeled ligand displaces half the specific binding of radiolabeled 17β -estradiol to the ER (IC_{50}). The K_d ,

which is measured in concentration units, is the equilibrium dissociation constant of the 17 β -estradiol-ER complex and represents the concentration of 17 β -estradiol that will bind to half the binding sites at equilibrium in the absence of competitors. A low K_d represents high affinity and a high K_d represents low affinity. The K_i is the analogous constant for the unlabeled ligand. The IC_{50} values depend on a number of factors, such as the specific assay system used, binding affinity of the unlabeled ligand for the ER, labeled 17 β -estradiol concentration, ER concentration, and experimental conditions (e.g., pH, exposure duration). In *in vitro* ER binding assays, there are substances that, because of biological inactivity, low solubility, or other considerations, do not decrease the binding of labeled, bound 17 β -estradiol by at least 50%. The IC_{50} values for these substances are often reported as being greater than the highest concentration tested or they are classified as “non-binders.” In this BRD, such substances are classified as negative in the ER binding assay conducted.

Because of the potential for variation in IC_{50} values among ER binding assays or repeats of assays that use different preparations of ER protein, the generally accepted method for presenting and comparing assay results is to compute the relative binding affinity (RBA) of the test substance against a reference estrogen. The RBA is calculated as $IC_{50}(\text{reference estrogen})/IC_{50}(\text{test substance}) \times 100$. 17 β -Estradiol is generally used as the reference estrogen for calculating the RBA value, but diethylstilbestrol (DES) has also been used. Because RBA values cover approximately eight orders of magnitude and because there is no current guidance as to which levels of activity are biologically meaningful in terms of an adverse health outcome, there is no general agreement regarding the distinction between the values needed to distinguish endocrine disruptors from non-disruptors.

1.2.3 Mechanistic Basis of *In Vitro* ER Binding Assays

The ER is a transcriptional regulatory protein belonging to the nuclear hormone receptor superfamily. The receptor is localized in the soluble nuclear fraction of estrogen target cells and plays a major role in controlling the transcriptional activation and/or repression of estrogen-responsive genes. The ER contains two discrete domains that are necessary for its role as a transcription factor – a ligand-binding domain in the C-terminal region and a DNA-binding domain in the N-terminal region of the protein. The ligand-binding domain, which is contained

within a wedge-shaped cavity on the receptor, is relatively hydrophobic. This allows the ligand-binding domain to accommodate its endogenous, non-polar ligand, 17 β -estradiol. The DNA-binding domain contains a zinc finger motif found in many DNA-binding proteins (Kumar et al., 1987; Brzozowski et al., 1997).

Recently, a second subtype of the ER, termed ER β , has been identified (Kuiper et al., 1997). The classical ER is now termed ER α . Many similarities exist between the two subtypes. The DNA-binding domains have about 97% amino acid homology, while the ligand binding domains have about 60% homology (Kuiper and Gustafsson, 1997). Because of these similarities, ER α and ER β share similar binding kinetics for many but not all of the estrogenic compounds tested with both subtypes. The two subtypes have unique tissue distributions, different physiological roles, and differ in their modes of regulating gene transcription (Kuiper et al., 1998; Gaido et al., 1999).

As the primary receptor for endogenous estrogens that initiate the transcription of messenger RNA and ultimately protein synthesis in estrogen-target cells, the ER plays a pivotal role in the development and maintenance of the female reproductive system. The interaction of estrogens with the ER in a cell initiates a cascade of events, including the dissociation of co-repressor proteins from the ER and the induction of significant, conformational changes in the receptor that allow the binding of co-activator proteins. This activated receptor complex binds to specific DNA regulatory sequences of estrogen-responsive genes (estrogen response elements; ERE) that are located upstream from or within the intron regions of the responsive genes. This binding initiates or inhibits the transcription of estrogen-controlled genes, which leads to the initiation or inhibition of cellular processes, respectively, including those necessary for cell proliferation, normal fetal development, or adult homeostasis (Kumar et al., 1987; Brzozowski et al., 1997; Love et al., 2000).

The current hypothesis for ER-mediated endocrine disruption is that certain xenobiotic substances, by virtue of their structure or conformation, bind to the ER and either mimic or block the action of 17 β -estradiol. The ER system is a prime candidate for interference by xenobiotic substances because the ligand-binding domain of the ER is much larger than the space occupied

by 17 β -estradiol, making the binding site somewhat nonspecific. This nonspecificity has been confirmed by studies demonstrating that a variety of different xenobiotic substances belonging to many structural classes bind to the ER (Blair et al., 2000; Pike et al., 2000; Fang et al., 2001). In addition, some substances, known as selective ER modulators (SERMs), cause the receptor to take on a conformation that is neither fully active nor inactive. SERMs have the ability to act as agonists in some estrogen-responsive tissues and as antagonists in others (McDonnell, 1999).

Potential agonist or antagonist estrogenic activity may be inferred for a substance by its ability to compete with 17 β -estradiol for binding to the ER. *In vitro* ER binding assays have been proposed as predictors of estrogen disruption in intact organisms (U.S. EPA, 1997; 1998a,b; 1999). The validity of the binding assay results for this purpose requires a determination that the substance also elicits similar responses in an *in vivo* assay. Such concordance for several substances has been reported by Shelby et al. (1996).

Factors that affect ligand binding to the ER are:

- Affinity for the ER. This affinity depends on the rates of the association and disassociation of the ligand with the receptor. The natural ligand, 17 β -estradiol, has a low equilibrium constant because of its rapid association rate and relatively slow disassociation rate. The half-life of the disassociation of 17 β -estradiol with the ER in intact rat uterine cells has been reported to be 90 minutes (Kassis et al., 1986).
- Systemic half-life of the ligand. This half-life will depend on its rate of metabolism to an intermediate that binds or does not bind to the receptor, and to the clearance of the ligand and its metabolites from the organism.
- Concentration of the ligand. Weakly binding ligands can produce a biological effect if they are administered at high enough concentrations, and strongly binding ligands would be ineffective if they do not reach estrogen-sensitive tissues.

1.2.4 Relationship of Mechanisms of Action in *In Vitro* ER Binding Assays Compared to the Species of Interest

Although the ER system is highly conserved among vertebrate species, and substances binding to ER derived from one species are expected to bind to the ER from another vertebrate species, the

relative binding affinities of these receptors for the same ligand may be different. Currently, little is known about the comparative binding of ligands to the ER of different species (Ankley et al., 1998). However, the ER from the rainbow trout has been reported to differ both structurally and functionally from its counterpart in mammals (Petit et al., 1995). In this regard, Zacharewski and co-workers (Matthews et al., 2000; Matthews and Zacharewski, 2000) recently showed that polychlorinated biphenyls (PCBs) have more affinity for the binding domain of rainbow trout ER (rtER) than to the binding domains of ERs from human, rat, mouse, or amphibian. Despite these differences and due to a lack of information on interspecies comparisons, the present working hypothesis is that the biological effects in one vertebrate species resulting from exposure to an endocrine disruptor is presumed to occur in other species. This approach is the basis for the use of ER binding as a general screen for estrogenic effects. The most widely used ER binding assays use human or rat ER-containing cells, or cytosolic ER derived from human or rat cells or tissues. Substances that bind the ER from these cells and tissues are presumed to be capable of producing estrogenic effects in multiple species. However, there is insufficient evidence to demonstrate that this extrapolation is appropriate. It is also not known whether differences in ER ligand affinity between species are meaningful with regard to *in vivo* adverse effects.

1.3 Intended Uses of the Proposed *In Vitro* ER Binding Assays

In vitro ER binding assays are proposed components of the EDSP Tier 1 screening battery. The Tier I battery is comprised of multiple *in vitro* and *in vivo* assays that assess both receptor- and non-receptor-mediated mechanisms of action and endpoints. This battery is designed to detect substances that might affect estrogen, androgen, and thyroid hormone systems in multiple species, including humans.

1.3.1 Validation of *In Vitro* Assays

The FQPA requires the U.S. EPA base its endocrine disruptor screening program on validated test systems, and that the assays selected for inclusion in the program be standardized prior to their adoption. The ICCVAM Authorization Act (Sec. 4(c)) mandates that “[e]ach Federal Agency ... shall ensure that any new or revised ... test method ... is determined to be valid for its proposed use prior to requiring, recommending, or encouraging [its use].” (P.L. 106-545,

2000). The validation process will provide data and information that will allow the U.S. EPA to develop guidance on the development and use of functionally equivalent assays and endpoints prior to the implementation of the screening program.

Validation is the process by which the reliability and relevance of an assay for a specific purpose are established (ICCVAM, 1997). Relevance is defined as the extent to which an assay will correctly predict or measure the biological effect of interest (ICCVAM, 1997). For the *in vitro* ER binding assays described in this BRD, relevance is restricted to how well an assay identifies substances that are capable of binding to the ER. The reliability of an assay is defined as its intra- and inter-laboratory reproducibility. Both relevance and reliability should be based on a diverse set of substances representative of the types and range of responses expected to be identified.

The first stage in assessing the validation status of an assay is the preparation of a BRD that presents and examines the available data and information about the assay, including its mechanistic basis, proposed uses, reliability, and performance characteristics (ICCVAM, 1997). This BRD summarizes the available data and information on the various types of *in vitro* ER binding assays that have been commonly used to characterize substances as potential endocrine disruptors. Where appropriate data are available, the qualitative and quantitative performance characteristics of the assays are evaluated, and the reliability of each assay is compared with the reliability of the other assays. These evaluations are used to determine whether a specific assay or assay type (e.g., whole cell, cell cytosol, tissue cytosol, recombinant ER) has been validated sufficiently to allow its recommendation for adoption by the U.S. EPA as an EDSP Tier 1 assay. If there are insufficient data to support the recommendation of an assay, this BRD will aid in identifying which specific assays should undergo further development or validation. The analyses can also be used to identify minimum procedural standards that should be considered for current and future *in vitro* ER binding assays.

1.3.2 Where Can *In Vitro* ER Binding Assays Substitute, Replace, or Complement Existing Methods?

There are no *in vitro* assays for ER binding or TA that are currently accepted by regulatory agencies. The *in vitro* ER binding assays are intended, along with other *in vitro* and *in vivo* tests, to be a component of the proposed EDSP Tier 1 battery for identifying endocrine disruptors.

1.3.3 Similarities and Differences with Currently Used Methods

The measurement of ER binding activity *in vitro* is not currently required for regulatory decision-making. However, there are a number of *in vitro* assays available for measuring receptor binding. These assays are based on the same general principles, but may use different sources of ER and different protocols.

The most frequently used ER binding assays use uterine cytosol from rats and mice as the source of the ER. Cytosol from other sources, such as the breast cancer cell line MCF-7, has also been used. Assays using purified receptor proteins have recently been introduced following the identification of the ER and ER receptors from different tissues. Relative binding of a ligand with the receptor has been measured using either radiolabeled 17 β -estradiol or by FP techniques.

1.3.4 Role of *In Vitro* ER Binding Assays in Hazard Assessment

The *in vitro* ER binding assays are proposed as a component of the proposed EDSP Tier 1 screening battery that also includes androgen receptor binding assays, *in vitro* ER and AR TA assays, and *in vivo* assays for endocrine effects in rodents, amphibians, and fish. The EDSTAC committee recognized that TA assays provide more information than binding assays because they measure also the consequences of binding. However, the limited databases at that time did not allow a determination of whether one or the other, or both assays, were preferred for screening (U.S. EPA, 1998a). Subsequently, the EDSP expressed a preference for TA assays over receptor binding assays because these assays can distinguish agonists from antagonists, and can be conducted with and without exogenous metabolic activation (U.S. EPA, 1999).

The assays in the Tier 1 battery have been combined in a manner such that limitations of one assay are complemented by strengths of another. The *in vitro* assays measure the interactions

between the test substance and binding and/or transcriptional activation only, and may therefore produce false positive results that may not occur *in vivo* due to limited absorption, distribution, metabolism, and excretion of the substance. The *in vitro* assays may also produce false negative results due to the absence of active metabolites that are formed *in vivo*, and to endocrine-related effects that are mediated by mechanisms not addressed by the *in vitro* assays.

A positive result in the ER binding assay or in other Tier 1 screening assays would not be sufficient to make the determination that a substance would produce a hormone-related adverse health effect in humans or other species. A weight-of-evidence approach will be used to evaluate the battery of Tier 1 results and to make decisions about whether or not a test substance would be subject to Tier 2 testing (U.S. EPA, 1998b). The Tier 2 assays are all performed *in vivo* and were selected to determine if a substance identified in Tier 1 as a potential endocrine disruptor exhibits endocrine-mediated adverse effects in animals and to identify, characterize, and quantify these effects.

1.3.5 Intended Range of Substances Amenable to *In Vitro* ER Binding Assays and/or Limits of *In Vitro* ER Binding Assays

The range of substances amenable to testing in *in vitro* ER binding assays has yet to be determined and will depend on the outcome of an independent peer review of the assays considered in this BRD. The *in vitro* ER binding assays are intended to be used to test food components and contaminants, as described in the FQPA (P.L. 104-170), and water contaminants, as described in the 1996 Amendments to the SDWA (P.L. 104-182). In addition, the U.S. EPA has authority to test commercial substances regulated by the Toxic Substances Control Act (TSCA, 1976) in the following circumstances: 1) the SDWA provides for testing of TSCA substances present in drinking water; 2) the FQPA amendments and the Federal Food Drug and Cosmetic Act (FFDCA; 21 CFR Ch.9., 1996) provide for testing of “inerts” in pesticide formulations; and 3) the FQPA and FFDCA provide for testing of substances that “act cumulative to a pesticide.”

1.4 Search Strategy and Selection of Citations for the *In Vitro* ER Binding BRD

The *in vitro* ER binding data summarized in this BRD are based on information found in the peer-reviewed scientific literature. An online literature search of entries in MEDLINE, CANCERLIT, TOXLINE, AGRICOLA, NIOSHTIC, EMBASE, CABA, BIOSIS, and LifeSci was conducted to retrieve database records on publications reporting on *in vitro* testing of substances for their endocrine disrupting effects. The search was conducted in the database basic index, which includes words in the title and abstract, and indexing words. Specifically, records on estrogen/androgen receptor binding assays and estrogen/androgen TA assays were sought. The search strategy involved the combining of “*vitro*” with alternative terms for estrogens, androgens, receptors, binding, and testing. Each database record included authors, bibliographic citation, and indexing terms. Most records also included abstracts.

Of the 459 records obtained from the initial search conducted on December 12, 2000, 354 contained data from estrogen-related assays and 105 contained data from androgen-related assays. Abstracts of selected titles were reviewed, and the relevant articles were selected and retrieved from the literature for analysis. A database of the literature citations was established using bibliographic database software. Subsequent to the initial search, additional articles with relevant information were identified and retrieved; many of these were identified from the bibliographies of the previously selected articles. Scanning of the literature using *Current Contents* and the British Lending Library’s *Table of Contents* continued through the writing of this BRD, and recently published articles were added to the database as they became available. Identification of ER-related publications for data extraction was completed on September 30, 2001.

The most relevant reports were those containing data on substances that have been tested in more than one laboratory using identical or related protocols. Every effort was made to include data from these publications because they provided information that could contribute to the assessment of the performance and reliability of the different assays. Publications containing data for substances that were synthesized specifically for the reported study and were not tested in other laboratories or in other *in vitro* ER binding assays did not contribute to the analysis of the data for performance and reliability. Primarily, these studies compared the binding affinities

of structural and positional isomers of known binding agents (such as 17 β -estradiol) that were synthesized specifically for the study and are not available commercially. Data on the ER binding affinity of some of these substances are included in the BRD. Data was not extracted from reports of studies using a unique procedure or from studies that tested obscure or difficult-to-identify substances. Based on these criteria, data from 72 publications was abstracted and included in this BRD.

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2.0 *IN VITRO* ER COMPETITIVE BINDING ASSAY METHODS

2.1 Introduction

The basic procedures to measure test substance binding to the ER were developed between 1965 and the early 1970s (Clark and Gorski, 1969). Cells containing an ER, semi-purified ER, or cytosolic fractions from cells containing an ER (typically from the uterus) are treated with sufficient amounts of radiolabeled reference estrogen (generally ^{125}I -estradiol) to saturate all of the ER binding sites. Following this treatment, the cells, proteins, or cellular extracts are challenged with the test substance, and the amount of radioactive reference estrogen remaining bound to the ER is measured by scintillation counting. The amount of bound radiolabeled reference estrogen is a function of the receptor-binding capacity of the test substance and the test substance concentration. Recently, a technique known as FP, in which a fluorescent estrogen molecule replaces the radiolabeled reference estrogen has been developed. In this assay, changes in the polarization of light are measured rather than scintillation counting of the amount of ER-bound radiolabeled reference estrogen.

Results from these competition assays are expressed as the K_i or as the IC_{50} . The K_i is a function of the affinity of the test substance and the radiolabeled reference estrogen for the ER. Despite the fact that the IC_{50} is very sensitive to experimental conditions while the K_i is less sensitive to these conditions, the majority of investigators present their data as IC_{50} values. This may be due to the fact that the most commonly used approach for comparing data within and between laboratories is the RBA, which is based on IC_{50} values.

The basic procedure proposed by Hähnel (1971) and Korenman (1970), among others, to measure ER binding has been modified over the years by numerous investigators. Common modifications include the source of the ER, the exposure duration and temperature, the reference estrogen, and the adsorbent used to separate the bound, radiolabeled estrogen from unbound molecules. For the purpose of summarizing the available ER binding assay approaches used by different investigators (**Appendix A**), the various protocols have been sorted according to whether they were performed with intact cells, a cell cytosol preparation, or with semi-purified preparations of human or rat ER or ER proteins. The protocols using cytosol have been further categorized according to the source of the cytosol (i.e., rat, mouse, or rabbit uterine

cytosol and cytosol from MCF-7 cells). The data generated from studies using a glutathione construct of the ligand binding domain (def) of the ER from humans and mouse, and the ER from a lizard (anole), chicken, and rainbow trout were categorized separately.

The first step in an ER binding assay is to determine the K_d of the reference estrogen (e.g., 17 β -estradiol) to the ER preparation used in the assay. The purpose of determining the K_d for each ER assay system is to demonstrate that the assay system is valid (e.g., a finite number of high affinity receptors are saturated with ligand) and to optimize the system with respect to receptor and ligand concentration. The K_d is determined in a saturation binding experiment that involves adding increasing concentrations of the radiolabeled reference estrogen to the cells/cytosol and measuring the amount that binds to the ER (Motulsky, 1995). To calculate specific binding of the radiolabeled reference estrogen to the ER, nonspecific binding (i.e., binding to sites other than ER) is measured at each radioligand concentration by the addition of a nonlabeled estrogen at a concentration that occupies all available receptors. The nonspecific binding is subtracted from the total binding (in the absence of nonlabeled compound) of the radiolabeled reference estrogen (Motulsky, 1995). The amount of radioligand specifically bound depends on the number (concentration) of receptors in the preparation. Free and bound radiolabeled ligands are separated by the addition of a non-reactive absorbent, such as dextran-charcoal or HAP. The ER, the bound radiolabeled reference estrogen, and other proteins in the reaction mix bind to the absorbent, while the displaced radiolabeled reference estrogen remains in the supernatant. The mixture is centrifuged and the amount of ER-bound radiolabeled reference estrogen in the pellet is measured. The K_d of the reference estrogen, which reflects its affinity for the specific ER preparation, can then be calculated. The K_d is used to determine the appropriate concentration of the reference estrogen to be used in the competitive binding assay.

Because the largest proportion of the published data was derived from studies using uterine cytosol from rats and mice, a general guideline for this method is described first. This guideline is followed with less detailed descriptions of other assays used to measure ER binding. In addition, copies of protocols were requested from investigators using the different *in vitro* ER binding assays; copies of the protocols received for public distribution are provided in **Appendix B**.

2.2 General Overview of *In Vitro* Assays Used To Measure Competitive ER Binding

The primary purpose of most *in vitro* ER binding studies was to investigate the nature of the binding process and the kinetics of the reaction, and to identify which molecular moieties enhanced or inhibited binding to the ER. Thus, most studies were not conducted to specifically identify endocrine disruptors. The publications presenting the results of relevant studies provided various levels of detail on the methods used, ranging from highly specific protocols to a simple listing of the ER source and the identity of the test substances.

A general protocol using uterine cytosol from rats and mice is described below, followed by less detailed descriptions of other assays used to measure ER binding. The majority of the *in vitro* ER binding studies considered for this BRD used 17 β -estradiol as the reference estrogen and, thus, this estrogen is included in the general protocols described in the following sections.

2.2.1 Mammalian Uterine Cytosol (Rat, Mouse, Rabbit) as the ER Source

Uterine cytosol is prepared by homogenizing the uterus in cold Tris buffer in a 1:10 ratio of tissue to buffer. The homogenate is centrifuged for 10 minutes at 2,500 x g at 4°C and the pellet containing cell debris is discarded. Next, the supernatant is centrifuged at 105,000 x g for 60 minutes at 4°C to pellet organelles and the cell cytosol supernatant containing the ER is stored at -70°C. Cytosolic protein concentration is determined using conventional methods.

To determine the K_d of 17 β -estradiol, radiolabeled (i.e., with ^3H) 17 β -estradiol at concentrations ranging from 1.0×10^{-8} to 3.3×10^{-11} M in buffer is added to aliquots of cytosol. Nonspecific binding of the radiolabeled 17 β -estradiol is measured at each concentration by the addition of nonlabeled 17 β -estradiol at a concentration that occupies all available receptors. Specific binding to the ER is then calculated at each concentration by subtracting nonspecific 17 β -estradiol binding from the total binding of 17 β -estradiol. After incubation and separation of bound and unbound radiolabeled 17 β -estradiol, the amount of radiolabeled 17 β -estradiol bound to the ER is measured. Specific binding data from saturation assays are usually analyzed to obtain the number of binding sites in a cytosolic preparation, B_{max} , and the K_d by nonlinear

regression using log concentration of radiolabeled 17 -estradiol as the independent variable (Motulsky, 1995).

The saturation binding curve of radiolabeled 17 -estradiol can also be analyzed using a linear Scatchard analysis (Scatchard, 1949) with specific binding on the abscissa (usually labeled “Bound”) and the ratio of specific binding of 17 -estradiol to free 17 -estradiol (usually labeled “Bound/Free”) on the ordinate. In these plots, B_{\max} is the x-intercept and K_d is the negative reciprocal of the slope. However, the Scatchard plot is not the most accurate technique to use for analysis because the data is transformed to make a linear graph that is then analyzed by linear regression, and transformation of the data distorts the experimental error. Linear regression analysis assumes that the scatter of points around a line follows a Gaussian distribution so that the standard deviation is the same at every value of X. However, this is not true with transformed data. Secondly, a Scatchard transformation alters the relationship between the “Bound” (X) and the “Bound/Free” (Y) ratio. This is because the value of X is used to calculate the value of Y and this calculation violates the assumptions of linear regression. Thus, the Scatchard values for B_{\max} and K_d are often further from their true values than if they had been calculated using nonlinear regression.

To measure competitive binding, radiolabeled 17 -estradiol, at a concentration that approximates the K_d , is added to tubes containing aliquots of the cytosol. A range of concentrations of the test substance in solvent, usually ethanol or unlabeled 17 -estradiol, is added. Nonspecific binding of radiolabeled 17 -estradiol to the receptor is measured by using a 100-fold molar excess of unlabeled 17 -estradiol. Following incubation of the mixture, the displaced radiolabeled 17 -estradiol is separated from the receptor-bound radiolabeled 17 -estradiol using an absorbent, such as dextran-charcoal or HAP. The radiolabeled 17 -estradiol-ER complex and the test substance-ER complex bind to the absorbent, and the unbound radiolabeled 17 -estradiol and test substance are removed by extensive washing of the absorbent. After centrifugation, the bound radiolabeled 17 -estradiol in the pellet is extracted with ethanol and the concentration of radiolabel is determined by scintillation counting. Specific binding is calculated by subtracting the amount of nonspecific binding from each sample evaluated in the assay. Data for the binding of the radiolabeled 17 -estradiol and its displacement by each test substance are plotted as the

percentage of radiolabeled 17 β -estradiol bound versus the molar concentration of competing test substance. For a substance with high affinity for the receptor, the upper plateau of the curve

$$\text{Specific binding} = \frac{B_{\max} \times [\text{Free radiolabeled } 17\beta\text{-estradiol}]}{K_d + [\text{Free radiolabeled } 17\beta\text{-estradiol}]}$$

correlates with maximal receptor binding in the absence of the test substance, and the bottom of the curve is the nonspecific binding. The concentration of the test substance that produces radiolabeled 17 β -estradiol binding half way between the upper and lower plateaus is the IC₅₀. Estimates of the IC₅₀ can be determined using appropriate statistical software.

The K_i, which reflects the affinity of the test substance for the ER, can be calculated from the IC₅₀ value using the equation of Cheng and Prusoff (1973):

$$K_i = \frac{IC_{50}}{1 + \frac{[\text{Radiolabeled } 17\beta\text{-estradiol}]}{K_d}}$$

The RBA value for each competing test substance is calculated by using the following equation:

$$RBA = \frac{IC_{50} \text{ for } 17\beta\text{-estradiol}}{IC_{50} \text{ for test substance}} \times 100$$

2.2.2 MCF-7 Cells and MCF-7 Cell-derived Cytosol

2.2.2.1 Intact MCF-7 Cells as ER Source

A number of cell lines inherently contain ER. The cell line most widely used for evaluating ER binding is the human breast adenocarcinoma cell line MCF-7. These cells are maintained in standard growth medium. Prior to their use in ER binding assays, the cells are grown for one to two days in medium containing charcoal-stripped serum. The purpose of charcoal stripping is to remove residual estrogenic substances that may competitively interfere with the binding of reference estrogens and test substances to the receptor.

For testing, intact cells are washed and treated with the radiolabeled ^{125}I -estradiol in serum-free minimal medium. Unlabeled test substances, including ^{125}I -estradiol, are added to the cells under non-growth conditions. Following incubation, the unbound test substance and reference estrogen are removed by washing the cells with ethanol. Scintillation counting is used to determine the extent of binding of the labeled reference estrogen. The amount of radiolabeled reference estrogen displaced by the test substance is used as the measure of its binding affinity for the ER.

2.2.2.2 MCF-7 Cytosol

A cell-free (cytosolic) extract of MCF-7 cells, which is prepared in a similar manner to cytosolic extracts from the rodent uterus, has been used as a source of ER. Cultured MCF-7 cells are harvested, homogenized to disrupt the cell membranes, and centrifuged to separate the nuclear debris and organelles from the cytosol. Generally, the assay is performed as outlined for the uterine cytosol assay.

2.2.3 Semi-Purified ER α and ER β

In the past few years, researchers have recognized the advantages of using molecular techniques to isolate the ER from mammalian tissues or to clone the DNA coding for the receptor into a plasmid, transfect a cell with the plasmid, and express the ER in a cell. The protein can be isolated and purified, or the cellular extract can be processed such that a semi-purified ER is obtained. Transfected cells or other cell lines with DNA transcripts from different species that code for the complete ER or for selected domains of the ER have been constructed.

Different approaches have been used to produce semi-purified ER α and ER β proteins for use as receptors to measure binding. To produce these proteins, the cDNA of the corresponding ER genes are cloned into a baculovirus or a transfer vector. The recombinant baculovirus vector is amplified and used to infect insect Sf9 cells (Bolger et al., 1998). Two days after infection, the cells are harvested and nuclei are isolated. A nuclear extract is made with buffer and the concentrations of ER proteins determined based on the specific binding of ^{125}I -estradiol to the receptors in solution (Kuiper et al., 1998). The semi-purified ER preparation compares favorably to the ER isolated from tissue preparations with respect to size, immunogenicity, hormone

binding characteristics, phosphorylation state, and DNA interactions in gel shift assays. This ER also interacts normally with its DNA response element (Cheskis et al., 1997; Ozers et al., 1997).

In some approaches, the cDNA of the ER protein is transcribed, whereas in others, only the cDNA coding for the ligand-binding domain of the ER protein is specifically excised and cloned, and the partial protein is expressed for use in the assay. Another approach has been the use of a rabbit cell expression lysate to produce the ER and ER proteins. The semi-purified ER proteins are produced by cloning the receptor genes into a plasmid, followed by the synthesis of the protein using the TnT-coupled reticulocyte lysate system with T7-RNA polymerase. Aliquots of the translation reaction mixture are used in the competitive binding assay (Kuiper et al., 1997).

2.2.3.1 Solid Phase Ligand Binding Assay using ScintiStrip™ (Kuiper et al., 1998)

The wells of ScintiStrip microtiter plates have scintillation fluors incorporated into the plastic. Signal detection is based on the premise that tritium (^3H) is a weak emitter and low energy electrons have a short range in solution; the tritiated molecules, binding to the solid support containing the fluor, will trigger a response. The assay is performed by binding the ER and ER proteins to the plastic support, followed by the addition of radiolabeled 17 β -estradiol and the test substance. Scintillation counting of the wells will detect only the radiolabeled 17 β -estradiol that remains bound to the ER, whereas radiolabeled 17 β -estradiol displaced from the ER by the test substance will not be detected.

2.2.4 GST-ERdef Fusion Proteins

GST-ERdef fusion proteins contain only the ligand binding domain (known as the def domains) of the ER fused to glutathione-S-transferase (GST). The def domains have been transcribed and translated for use in measuring the ER-binding of 17 β -estradiol and other substances. This approach has been used to prepare the partially purified binding domains of the ER protein from the lizard (anole), chicken, and rainbow trout, and the ER from human and mouse (Matthews et al., 2000; Matthews and Zacharewski, 2000).

Essentially, assays using GST-ERdef proteins are performed as described above for cytosol except incubations are in 1 mL glass tubes arranged in a 96-well format. Bound radiolabeled 17 β -estradiol is separated from free radiolabeled 17 β -estradiol using a 96-well filter plate and vacuum pump harvester. The filter plates containing the protein are washed with buffer and the plates are allowed to dry under continuous suction. After drying, the undersides of the filter plates are sealed and scintillation cocktail is added to each well. Bound radiolabeled 17 β -estradiol is measured using a scintillation counter. Nonspecific binding of 17 β -estradiol is determined in the presence of a 400-fold excess of unlabeled 17 β -estradiol (Matthews and Zacharewski, 2000).

2.2.5 Fluorescent Polarization (FP)

2.2.5.1 Theory of FP

FP is a technique that can detect molecular interactions by monitoring changes in the size of fluorescently labeled or inherently fluorescent molecules (Dandliker et al., 1981; Checovich et al., 1995; Jameson and Sawyer, 1995; Lundblad et al., 1996). When a fluorescent molecule binds to another molecule, its speed of rotation changes. This change in speed or tumbling rate can be quantified by FP. When a solution of fluorescent molecules is excited by plane-polarized light, those molecules parallel to the plane become excited. If the molecules remain stationary during the period of excitation (4 nanoseconds for fluorescein), the emitted light remains highly polarized. However, if the molecules tumble during the period of excitation, the emitted light will be random or depolarized. An increase in the volume or conformation of a fluorescent molecule (e.g., through its binding to a receptor or antibody) or a decrease in its conformation or molecular volume (due to dissociation or enzymatic degradation) can be directly measured by FP. The observed value is a weighted average of the polarization values of the individual bound and free fluorescent molecules, and is therefore a direct measure of the fraction bound. The concentration of the bound ligand is derived from the polarization value, and the resultant bound versus free isotherm is analyzed in a similar manner to the graph generated by conventional techniques for radioactivity (Dandliker et al., 1981; Checovich et al., 1995; Jameson and Sawyer, 1995).

For the FP assay, purified, full-length, hER or and an intrinsically fluorescent non-steroidal estrogen (Fluormone ES1; FES1), which binds to the hER with high affinity, are used (Bolger et al., 1998). This particular fluorescent estrogen was developed by Katzenellenbogen and colleagues (Hwang et al., 1992).

In the competitive binding assay, substances are tested for their ability to displace the fluorescent ligand FES1 from an ER-FES1 complex. The large ER-FES1 complex tumbles slowly and therefore has a high anisotropy value. As increasing concentrations of a competing ligand displace the FES1 from the complex, the free FES1 molecules tumble more rapidly and have a lower anisotropy value. As more FES1 molecules are displaced from the complex, the measured anisotropy approaches the free anisotropy value. The measured anisotropy is a weighted average of the bound and free FES1 molecules.

2.2.5.2 Conduct of the Assay

As described for the other assays, a binding constant of reference estrogen, in this case FES1, to the receptor must be determined. This approach ensures that a saturating concentration of FES1 is used in the competitive binding assay. The receptor is serially diluted and the same concentration of FES1 is added to each tube. After incubation at room temperature, the fluorescence anisotropy of each tube is measured with a 360 nm excitation filter and a 530 nm emission filter. The anisotropy at each ER concentration is converted to the fraction of ligand bound using the following equation:

$$F_b = \frac{A - A_f}{A_b - A_f}$$

where F_b is the fraction of ligand bound, A is observed anisotropy, and A_b and A_f are anisotropy values of the bound and free ligand, respectively.

Bound ER (ER_b) is assumed to be equal to bound ligand (L_b), and therefore determined by multiplying F_b by the total ligand concentration (L_t). Free ER (ER_f) is calculated by subtracting ER_b from the total ER in the assay (ER_t). The equilibrium binding constant, K_d , is calculated from the ER-bound versus ER-free isotherm using a nonlinear least-square curve fitting program.

In performing the competitive binding assay, aliquots of the serially diluted test compound are added to known concentrations of hER and FES1. Negative controls containing hER + FES1 (equivalent to 0% inhibition), and positive controls containing free FES1 (equivalent to 100% inhibition), in the absence of competitor, are included in each run. Varying concentrations of the competitive ligand are added to tubes containing the same concentrations of hER and FES1. After incubation at room temperature, the anisotropy value in each tube is measured. The anisotropy values are converted to percent inhibition using the following formula:

$$I\% = \frac{A_o - A}{A_o - A_{100}} \times 100$$

where A_o , A_{100} , and A are the percent inhibition, A_o at 0% inhibition, A_{100} at 100% inhibition, and the observed A value, respectively.

Polarization values are converted to percent inhibition to normalize day-to-day differences in the starting 0% inhibition polarization values. The percent inhibition versus competitor concentration curves is analyzed by nonlinear least-squares curve fitting to yield an IC_{50} value. IC_{50} values are converted to an RBA value using 17 β -estradiol as a standard; the RBA value using 17 β -estradiol is set to 100.

2.2.6 Permutations of the Assays as Described in the Literature

Irrespective of source of the ER used in a particular study, the protocols vary from laboratory to laboratory. Some of these variations are in response to the differing properties of the ER preparations used, or because of various questions the studies were designed to address. The permutations in the protocols used by each laboratory for each source of ER are summarized in **Appendix A**.

3.0 CHARACTERIZATION OF SUBSTANCES TESTED IN ER BINDING ASSAYS

3.1 Introduction

ER binding data were obtained for a total of 638 substances (**Appendix C**). While a relatively large number of substances have been tested in ER binding assays, only a small number of these substances were evaluated in multiple types of ER binding assays and/or by multiple laboratories. With the exception of 17 β -estradiol, the reference estrogen used in most studies, only 14 substances (2.2%) were tested in at least ten of the 14 assays considered in this BRD. These substances are bisphenol A, 2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), chlordecone, coumestrol, *o,p'*-dichlorodiphenyltrichloroethane (*o,p'*-DDT), diethylstilbestrol (DES), 5 α -dihydrotestosterone, estriol, estrone, 4-hydroxytamoxifen, genistein, methoxychlor, tamoxifen, and zearalenone. Ninety-four percent (600) of the substances in the database were tested in five or fewer assays, 63% (403) were tested in one assay only, and 59% (376) were tested in one publication only.

3.2 Rationale for Selection of Substances/Products Tested in *In Vitro* ER Binding Assays

Most of the substances tested in *in vitro* ER binding assays closely parallel the initial studies on the isolation and characterization of the receptor, the subsequent synthesis and characterization of ER agonists and antagonists, and the more recent use of ER binding assays as a method for endocrine disruptor screening. Many of the first substances to be tested were selected to address basic research questions regarding the nature of the ER and the kinetics of its interactions. A number of the triphenylethylenes, stilbenes, and DES analogs and derivatives, for example, were investigated to obtain a better understanding of ER binding processes. Some substances were investigated in research and development studies designed to determine which metabolite or stereoisomer of a molecule enhanced or inhibited binding to the ER. Data from these types of studies often contributed to the development of pharmaceuticals for breast cancer, estrogen-replacement therapy, or for other health concerns. Some substances were investigated to determine structure-activity relationships (SAR) for the development of quantitative SAR (QSAR) models. Finally, during the last decade, with the growing concern about possible adverse health effects associated with exposure to endocrine disruptors, some of these substances (e.g., pesticides, polychlorinated biphenyls, phytoestrogens) were tested using *in vitro* ER

binding assays to identify those that may act as estrogen agonists/antagonists in humans and wildlife.

3.3 Chemical and Product Classes Tested

Chemical and product class information for the substances tested in ER binding assays is provided in **Appendix C**. Substances were assigned to a single chemical class based on available information from standardized references (e.g., *The Merck Index 12th Edition* and the U.S. National Library of Medicine's ChemID database) and from an assessment of chemical structure. As shown in **Table 3-1**, the chemical classes with the greatest amount of *in vitro* ER binding data are polychlorinated biphenyls, phenolic and non-phenolic steroids, triphenylethylenes, organochlorines, polycyclic aromatic hydrocarbons, stilbenes, phenols, and bisphenols. Of the 638 substances included in **Appendix C**, seven substances were not classified within a chemical class.

Product classes were assigned based on information contained in *The Merck Index* and the U.S. National Library of Medicine's ChemFinder. As show in **Table 3-2**, the most common product classes tested in *in vitro* ER binding assays have been pharmaceuticals, pesticides, chemical intermediates, dielectric fluids or their components, natural products (including several phytoestrogens), and plasticizers. Of the 638 substances included in **Appendix C**, 320 were not classified within a product class.

Table 3-1 Chemical Classes Tested in *In Vitro* ER Binding Assays (638 Substances)

Chemical Class	# of Substances
Acetamide	2
Acrylate	6
Alcohol	4
Aldehyde	1
Alkoxyphenol	5
Alkylbenzene	2
Alkylphenol	14
Amide	1
Anilide	2
Aniline	4
Aromatic amine	1
Aromatic heterocycle	1
Aromatic hydrocarbon	1
Azo compound	1
Benzophenone	6
Biphenyl	3
Biphenyldiol	1
Bisphenol	27
Carbamate	3
Carboxylic acid	4
Chalconoid	6
Chlorinated aromatic hydrocarbon	1
Chlorinated bridged cycloalkene	2
Chlorinated cycloalkane	1
Chlorinated cyclodiene	4
Chlorinated phenol	4
Coumarin	1
Crown ether	1
Cyclodiene	1
Dioxin	1
Diphenolalkane	18
Diphenolalkene	4
Diphenyl ether	2
Diphenylalkane	5
Ester	1
Flavanone	10
Flavone	14
Glucuronide	1

Heterocyclic aromatic aldehyde	1
Imidazole	1
Indane	1
Indene	15
Isoflavone	15
Nitrobenzene	1
Nitrogen heterocycle	2
Organochlorine	44
Paraben	7
Phenol	40
Phenoxy carboxylic acid	1
Phosphate ester	1
Phthalate	13
Phthalimide	1
Piperidine	1
Polychlorinated biphenyl (PCB)	93
Polycyclic aromatic hydrocarbon	42
Polyether	1
Pteridine	1
Purine	1
Pyrazole	1
Pyrethrin	6
Pyrethroid	6
Resorcylic acid lactone	6
Siloxane	2
Steroid, nonphenolic	58
Steroid, phenolic	69
Stilbene	40
Sulfoxide	1
Terpene	2
Tetrahydrophenanthrene	1
Thiophene	2
Triazine	9
Triphenylethylene	49
Triphenylmethane	2
Not classified	7

Table 3-2 Product Classes Tested in *In Vitro* ER Binding Assays (638 Substances)

Product Class	Number of Substances
Analytical reagent	1
Antioxidant	1
Chemical additive	2
Chemical intermediate (adhesive, coatings, cosmetic, dye, elastomer, fiber, film, flavor, fragrance, lubricant, monomer, pesticide, plasticizer, pharmaceutical, polyester, polymer, resin, surfactant)	57
Dielectric fluid or component	54
Dye	4
Flavor	2
Food additive	16
Fragrance	6
Lubricant additive	1
Natural product (plant or animal)	31
Pesticide/Pesticide metabolite	61
Pharmaceutical/Pharmaceutical metabolite/ Pharmaceutical additive	92
Plant growth regulator	1
Plasticizer	16
Polymer	2
Preservative	5
Solvent	4
Surfactant	1
Not classified	320

4.0 REFERENCE DATA

The ability of a test substance to bind to the ER *in vitro*, whether to an isolated protein receptor molecule or to ERs in cultured cells, suggests, but does not demonstrate, the ability of the substance to act as an estrogen agonist or antagonist. A commonly used *in vitro* method to measure such biological effects is based on an assessment of the ability of a substance to induce or inhibit transcriptional activation of an ER-dependent reporter gene function.

The purpose of this BRD is to assess the sensitivity of various *in vitro* ER binding assays for detecting ER-binding substances with various binding activities and to assess reliability within and among laboratories and across procedures. No attempt is made to evaluate their performance with respect to other *in vitro* biological end points, such as transcriptional activation, or *in vivo*, such as promotion of uterine growth. Such comparisons will be addressed elsewhere. Therefore, no reference data are included for assessing the biological relevance of the ER binding assays.

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5.0 DATA ON *IN VITRO* ER BINDING ASSAYS

5.1 Introduction

Methods and ER binding data were collected from 72 publications reporting studies in which the competitive binding of a substance to the ER was measured and RBA values were included or could be calculated. When provided, the specific information extracted for each substance included its name, source, purity, methodological details, relevant binding data (K_i , IC_{50} , and/or RBA values for positive studies, highest dose tested [HDT] for negative studies), and the citation. For studies in which chemical structures only were provided, every effort was made to identify the name of each substance tested. No attempt was made to identify the source and purity of a substance if the investigators did not provide such information. If available, a Chemical Abstract Service Registry Number (CASRN) was entered for each substance. This identifier was obtained from various sources, including the publication, the National Library of Medicine's ChemID database, and *The Merck Index*. Chemical name synonyms were entered for substances that were identified in the literature by more than one name, and for substances where the literature name may have been different from the generic name. All substances with the same CASRN were listed under the same name, regardless of the name that was used in the original publication. **Appendix C** provides information on the names, synonyms, CASRN, and chemical/product class, where available, for each substance, while **Appendix D** contains the *in vitro* ER binding data sorted alphabetically by substance name.

5.2 Availability of Detailed *In Vitro* ER Binding Protocols

The scientific methods presented in the publications containing data from competitive *in vitro* ER binding studies provided various levels of detail. To the extent possible, the most important method parameters were extracted from each publication and summarized in **Appendix A**. Details about the following method parameters are included in the Appendix to the extent this information was available:

- Preparation of the receptor (e.g., species or cell line, buffer used for preparation of cytosol, protein concentration of cytosol);
- Competitive binding assay (e.g., concentration of radiolabeled estrogen, solvent used to dissolve the test substance, concentration range of the test substance, number of replicates within an assay, number of times assay was repeated);

- Separation of ligand (e.g., type of slurry used, incubation time, temperature); and
- Data calculations (e.g., method used for calculating data, data format).

5.3 Availability of *In Vitro* ER Binding Data

ER binding data were collected for a total of 638 substances tested in competitive binding studies with ER obtained from the following sources:

1. Rat uterine cytosol (RUC);
2. Mouse uterine cytosol (MUC);
3. Rabbit uterine cytosol (RBC);
4. Cytosol from human adenocarcinoma MCF-7 cells (MCF-7 cytosol);
5. Intact MCF-7 cells (MCF-7 cells);
6. Semi-purified human ER protein (hER_α);
7. Semi-purified human ER protein (hER_β);
8. Semi-purified rat ER protein (rER_α);
9. Semi-purified human ER as measured by FP (hER_α-FP);
10. Glutathione-S-transferase fusion proteins consisting of the def domains of the human ER receptor (GST-hER_αdef);
11. Glutathione-S-transferase fusion proteins consisting of the def domains of the mouse ER receptor (GST-mER_αdef);
12. Glutathione-S-transferase fusion proteins consisting of the def domains of the lizard (anole) (GST-aERdef);
13. Glutathione-S-transferase fusion proteins consisting of the def domains of the chicken (GST-cERdef); and
14. Glutathione-S-transferase fusion proteins consisting of the def domains of the rainbow trout (GST-rtERdef).

In all studies, competitive binding was measured by the displacement of radiolabeled ($[^3\text{H}]$ or $[^{125}\text{I}]$) 17 β -estradiol from the ER-estrogen complex or by the change in anisotropy of the fluorescent ER-estrogen complex by the test substance. **Appendix D** presents the extracted and compiled data sorted first by substance name and then by assay. In those cases in which the RBA value was not provided in the citation, this value was calculated, when possible, from

provided IC₅₀ values. Not all of these values were reported in all publications. In some publications, neither the IC₅₀ nor the RBA values were presented. In many of these cases, the binding of the test substance to the ER over a range of concentrations was presented graphically, so that the IC₅₀ values of 17 β -estradiol and the test substance could be estimated. These estimated IC₅₀ values and corresponding calculated RBA values are italicized in **Appendix D**. For substances that did not bind sufficiently well to the ER to displace the reference estrogen (i.e., an IC₅₀ value could not be calculated), the only parameter that could be entered into the database was the HDT.

5.4 In Vitro ER Binding Assay Results for Individual Substances

The number of *in vitro* ER binding assays in which each substance was tested is provided in **Appendix E**. These data, shown in **Table 5-1**, are summarized by assay and ranked according to the number of substances tested. Of the 638 substances tested in the 14 different *in vitro* ER binding assays, the majority of substances (376 or 59%) had been tested in the RUC assay. Only 133 (21%) of these substances had been tested in the next most frequently used assay, hER . For five of the 14 assays (hER -FP, RBC, rER , GST-mER def, GST-cERdef), published data on less than 50 substances for each assay were located.

As presented in **Table 5-2**, only 14 (excluding the reference compound 17 β -estradiol) of the 638 substances (2.4%) had been tested in 10 or more assays, and of these, only three substances (0.47%) had been tested in all 14 assays. As stated in **Section 3**, 94% (600) of the substances in the database had been tested in one to five assays, with 63% (403) tested in one assay only.

Table 5-1 Number of Substances Tested in Various *In Vitro* ER Binding Assays (638 Substances)*

Assay	Number of Substances Tested	% of Total Substances Tested
RUC	376	59%
hER	133	21%
hER	101	16%
GST-hER def	99	16%
MCF-7 cytosol	94	15%
GST-rtERdef	86	13%
GST-aERdef	85	13%
MUC	75	12%
MCF-7 cells	66	10%
hER -FP	48	8%
RBC	45	7%
rER	37	6%
GST-mER def	34	5%
GST-cERdef	34	5%

*Assays sorted according to the number of substances tested.

Table 5-2 Substances Tested in Ten or More *In Vitro* ER Binding Assays*

Substance	Number of Assays	Number of Publications
17 -Estradiol	14	72
Diethylstilbestrol	14	30
Bisphenol A	14	22
Tamoxifen	14	13
Estrone	13	12
<i>p,p'</i> -Methoxychlor	13	11
4-Hydroxytamoxifen	13	10
<i>o,p'</i> -DDT	12	9
Estriol	12	9
Genistein	11	9
Coumestrol	11	7
Kepone	10	7
2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1-trichloroethane (HPTE)	10	7
5 -Dihydrotestosterone	10	6
Zearalenone	10	5

*Substances sorted by the number of assays tested and then by the number of publications.

5.5 Use of Coded Chemicals and Compliance with Good Laboratory Practice (GLP) Guidelines

Based on the available information in the scientific literature, it appears that the published *in vitro* ER binding assay studies neither used coded chemicals nor were they conducted in compliance with GLP guidelines (see **Section 8**).

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6.0 IN VITRO ER BINDING TEST METHOD PERFORMANCE ASSESSMENT

6.1 Introduction

The ICCVAM Submission Guidelines (ICCVAM, 1999) request that an assessment be conducted of the performance (i.e., accuracy, sensitivity, specificity, positive and negative predictivity, and false positive and false negative rates¹) of the proposed test method with respect to its ability to predict the effect of interest in the reference test method currently accepted by the regulatory agencies and, where feasible, to predict adverse health outcomes in the species of interest (e.g., humans, wildlife). Currently, there are no validated *in vivo* reference test methods developed to specifically assess the ability of a test substance to disrupt endocrine function, and data on endocrine disruption in humans or wildlife are too limited to be used for this purpose. Therefore, the existing *in vitro* ER binding assays were compared against each other with regard to their ability to detect substances capable of binding to the ER. However, this type of analysis of *in vitro* ER binding assays is limited by the lack of multiple test data within and across assays for most of the substances considered, and by the paucity of data for the same substances tested in multiple assays.

Taking these limitations into account, a comparative evaluation was conducted of the relative performance of the 14 *in vitro* ER binding assays considered in this BRD. Both quantitative and qualitative assessments of IC₅₀ and RBA values were conducted. The quantitative assessment was based on the 238 substances (37.3% of the 638 substances in the *in vitro* ER binding assay database) that had been tested in at least two assays (**Appendix E**), and was further limited to individual tests that resulted in an IC₅₀ or RBA value (i.e., the substance was classified as positive). The qualitative assessment was limited to the 100 substances that had been tested in the RUC assay and in at least one of the 13 other *in vitro* ER binding assays, and included substances classified as negative for ER-binding activity.

¹ Accuracy is defined as the proportion of correct outcomes of a method, often used interchangeably with concordance; Sensitivity is defined as the proportion of all positive substances that are correctly classified as positive in a test; Specificity is defined as the proportion of all negative substances that are correctly classified as negative in a test; Positive predictivity is defined as the proportion of correct positive responses among substances testing positive; Negative predictivity is defined as the proportion of correct negative responses among substances testing negative; False positive rate is defined as the proportion of all negative substances that are falsely identified as positive; False negative rate is defined as the proportion of all positive substances that are falsely identified as negative (NIEHS, 1997).

Table 6-1 Number of Substances Tested in Multiple *In Vitro* ER Binding Assays

Number of Assays	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Number of Substances	403	87	74	23	13	6	7	5	5	4	2	2	3	4	638
% of Substances	63.2	13.6	11.6	3.6	2.0	0.9	1.1	0.8	0.8	0.6	0.3	0.3	0.5	0.6	100

6.2 Quantitative Assessments of Assay Performance

To reduce the extent of skewness in the data prior to conducting the quantitative assessments, the two outcome variables for *in vitro* ER binding assays — the RBA and the IC₅₀ values — were transformed using the natural log. Studies that did not result in an IC₅₀ and/or RBA value were eliminated from consideration. Given the large number of data points for modeling, the general linear models (GLM) used in this analysis are robust, although some skewness may yet exist with the data. To simplify the comparison, each literature citation was considered an independent assessment (designated here as a ‘reference’).

Two-way and three-way analysis of variance models were performed with random effects to estimate the intra-class correlation of substances. A high correlation value indicates that the lnRBA or lnIC₅₀ values are more similar within groups than among groups, where groups can be defined by assay or by reference. Estimates of variance for each model component and intra-class correlation are presented to show which factors (substance, assay, or reference) are responsible for the greatest variation in the lnRBA and lnIC₅₀ values. Due to limitations in the database with regard to the number of substances tested in multiple assays and to the number of independent tests performed for the substance using the same assay, the results of these analyses must be viewed with caution.

Initially, all data representing all substances, assays, and references were considered, and unique data (i.e., substances tested only in a single assay) were excluded from subsequent analyses. For the analysis of lnRBA values, a total of 752 data points representing 211 substances, 14 assays, and 51 references were considered. For the analysis of lnIC₅₀ values, 369 data points representing 119 substances, 13 assays, and 31 references were considered. The lnIC₅₀ and the lnRBA values for 17 α -estradiol were omitted from these analyses. The RBA values for 17 α -

estradiol are uninformative because they are arbitrarily set at 100% in all assays in which this substance is used as the reference estrogen. The IC₅₀ values for 17 β -estradiol represent the largest collection of IC₅₀ data for a single substance and were evaluated independently to avoid potentially biasing the quantitative analysis.

6.2.1 Measures of Intra-Class Correlation

The intra-class correlation, r_I , measures the percentage of variation in y , the outcome variable, explained by a given component or set of components. The model is $y = \text{substance} + \text{assay} + \text{reference}$. **Table 6-2** contains the components of variance for each variable adjusted for the other two variables. Interpretation of this analysis is limited to factors that impact on performance; factors that impact on assay reliability are discussed in **Section 7**.

From this analysis, it appears that the lnRBA or lnIC₅₀ values for a specific substance were generally consistent irrespective of which assay was used or which laboratory conducted the study. The greatest variation in lnRBA or lnIC₅₀ values was found between substances (i.e., the most important parameter was the intrinsic ER binding property of the substance). The greater contribution of substances to the overall variance is not surprising considering the seven orders of magnitude range in reported IC₅₀, and thus RBA, values.

6.2.2 Evaluation of Substances Tested in Nine or More *In Vitro* ER Binding Assays

In this analysis, the variances for the RBA values of the 12 substances that had been tested in at least nine of the 14 *in vitro* ER binding assays were determined. Although 14 substances (excluding 17 β -estradiol) had been tested in at least ten *in vitro* ER binding assays (**Section 5**), only those substances that elicited a positive response in at least one experiment in each

assay could be used in this analysis. The variances and sample sizes for these 12 substances are provided in **Table 6-3**, ranked in descending order according to the median RBA value based on all test data. Only assays for which variances could be calculated are included, and most of these variances were based on three or four values only. Due to the lack of sufficient data, a corresponding analysis of IC₅₀ values was not conducted.

Table 6-2 Components of Variance for Each Variable Adjusted for the Other Two Variables – Performance Assessment

	Outcome, y (% variation)	
	lnRBA	lnIC ₅₀
Var(substance)	8.34	8.49
Var(assay)	0.38	0.34
Var(reference)	1.40	2.01
Var(error)	1.75	2.44
Corr (y _{ijk} , y _{ij'k'})*	0.70	0.64
Corr (y _{ijk} , y _{ijk'})**	0.73	0.67
Corr (y _{ijk} , y _{ij'k'} ***)	0.82	0.79

*A high correlation was found for the lnRBA values within substances using any assay or reference (i.e., the lnRBA values are more correlated within than across substances). A slightly lower correlation was found when lnIC₅₀ values were used. The high correlation for the lnRBA values suggests that the RBA of a specific substance to the ER did not vary much among the different binding assays.

**This correlation suggests that the test substances responded similarly in an assay irrespective of the laboratory in which the test was conducted. Variation within laboratories is slightly less than the variation across laboratories.

*** A high correlation was found for substances tested in the same laboratories (i.e., references) but using different assays.

A large p value (p1 or p2) identifies those substances, such as zearalenone, estriol, estrone, diethylstilbestrol (DES), 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), bisphenol A, and chlordecone, with the least amount of variability in their lnRBA values. In contrast, the p1 values of coumestrol and tamoxifen are below 0.05, indicating that significant variability exists across assays irrespective of the laboratory in which the tests were performed. A possible explanation for the variability with coumestrol, a phytoestrogen, is its ~1.5-log greater binding affinity to the ER α protein compared to the ER β protein (**Appendix D**). No explanation can be provided for the significant variability in lnRBA values for tamoxifen. Values for p2 could not be calculated in every case since there were too few assays or references that could be used in the

analysis. A significant p_2 value was not found for any substance suggesting that there was not significant variability due to the reference (i.e. laboratories in which the substance was tested).

Another approach to evaluating the variability across assays for a substance is to fit a two-way model, where $y = \text{assay} + \text{reference}$. In this analysis (**Table 6-4**), adjustment is made for inter-reference variation in lnRBA so that only those assays used twice or more in two or more

Table 6-3 Variance of lnRBA Values by Substance and Assay – Performance Assessment^a

Substance ^b (CASRN)	Median ^c RBA	#of Obs/ # Assays	hER α ^d	hER α - FP ^d	hER β ^d	MCF-7 cytosol ^d	MUC ^d	RUC ^d	p1*	p2**
4-Hydroxy- tamoxifen (68047-06-3)	168	18/13	0.28 (3)	1.82 (3)					0.08	0.15
DES (56-53-1)	127	38/14	0.99 (3)	0.45 (4)			0.60 (7)	3.42 (11)	0.15	0.99
Estrone (53-16-7)	45	18/13				2.40 (3)		0.98 (4)	0.73	na ^e
Estriol (50-27-1)	15.8	16/12				2.42 (4)			0.53	0.64
Zearalenone (17924-92-4)	15.0	11/9	All n \leq 2						0.42	na
Tamoxifen (10540-29-1)	5.0	21/14	0.44 (3)					2.01 (4)	0.02	0.10
Coumestrol (479-13-0)	3.1	15/11	0.79 (3)						0.02	0.25
HPTE (2971-36-0)	1.45	12/10						1.53 (3)	0.82	na
Genistein (446-72-0)	1.30	18/11	1.07 (4)		0.97 (3)				0.11	0.18
Bisphenol A (80-05-7)	0.031	22/14	1.36 (3)					1.25 (5)	0.53	0.60
<i>o,p'</i> -DDT (789-02-6)	0.038	15/10						1.72 (5)	0.20	na
Kepone (143-50-0)	0.027	11/9						1.39 (3)	0.60	na

^aOnly assays where a variance could be calculated for at least one of the 12 substances are listed. The variance for a particular assay could be calculated only if a particular substance was tested three or more times in that assay; empty cells indicate insufficient data to calculate a variance. The p values could be calculated only if there were two observations from at least three or more assays; a missing p-value indicates insufficient data.

^bSubstances that had been tested in at least 9 of the 14 *in vitro* ER binding assays; DES = diethylstilbestrol; *o,p'*-DDT = *o,p'*-dichlorodiphenyltrichloroethane; HPTE = 2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane.

^cThe median RBA value across assays, based on positive test data.

^dThe numbers in parenthesis indicate the numbers of replicate tests.

^ena = No p value could be calculated since there was either no values or only one value per assay x response combination.

*p1 tests whether there is a significant difference among all assays used; unadjusted for references.

**p2 tests whether there is a significant difference among all assays used; adjusted for references.

laboratories (references) are considered. Results are presented in descending order according to the median RBA value across assays, based on all positive test data, for each of the 12 substances. The components of the variance for each variable are adjusted for the other variable. Due to the lack of sufficient data, a corresponding analysis of IC₅₀ values was not conducted.

Table 6-4 Variance for Y=lnRBA Values

Substance ^a (CASRN)	Median RBA ^b	N ^c	n/n ^c	var(assay)	var(ref)	var(error)	r _I ^d (assay)
4-Hydroxy-tamoxifen (68047-06-3)	168	18	13/8	0.66	1.58	0.17	0.27
DES (56-53-1)	127	38	14/8	≤0.001	6.37	0.35	~0 ^e
Estrone (53-16-7)	45	18	13/7	0.25	2.88	0	0.08
Estriol (50-27-1)	15.8	16	12/7	0.096	4.54	0.49	0.001
Zearalenone (17924-92-4)	15.0	11	9/6	0.27	Too few references	0.44	0.38
Tamoxifen (10540-29-1)	5.0	21	14/8	0.53	1.91	0.08	0.21
Coumestrol (479-13-0)	3.1	15	11/7	0.49	0.22	0.43	0.43
HPTE (2971-36-0)	1.45	12	10/6	1.14	2.34	0	0.33
Genistein (446-72-0)	1.30	18	11/7	1.41	≤0.001	1.23	0.53
<i>o,p'</i> -DDT (789-02-6)	0.038	15	10/4	2.89	2.90	0	0.50
Bisphenol A (80-05-7)	0.031	22	14/8	≤0.001	≤0.001	2.64	~0
Kepone (143-50-0)	0.027	11	9/6	0.84	1.93	0	0.30

^aSubstances that had been tested in at least nine of the 14 *in vitro* ER binding assays; DES = diethylstilbestrol; *o,p'*-DDT = *o,p'*-dichlorodiphenyltrichloroethane; HPTE = 2,2-Bis(*p*-hydroxyphenyl)-1,1,1,-trichloroethane

^bThe median RBA value across assays, based on positive test data.

^cN is the total number of values available; n is the number of assays used to test that substance; and n' is the number of assays that can be adjusted for the effect of reference to generate the data in this table.

^dr_I, the intra-class correlation, measures the percentage of variation in y, the outcome variable, explained by a given component or set of components

^er_I= 0 when each RBA value is derived from a different assay x reference combination

As demonstrated by the relatively small intra-class correlation values, the lnRBA values are very similar across assays for estriol and estrone, and not quite as similar across assays for tamoxifen, HPTE, chlordecone, and 4-hydroxytamoxifen. The relatively large intra-class correlation values for genistein, coumestrol, *o,p'*-DDT and zearalenone suggest that these substances respond differently in the various assays. The explanation for the increased variability associated with genistein and coumestrol, both of which are phytoestrogens, might be their ~1.5-log greater binding affinity to the ER α protein compared to the ER β protein used in other assays. No explanation can be provided for the increased variability in lnRBA values associated with zearalenone and *o,p'*-DDT. However, the lack of an obvious relationship between the magnitude of the median RBA value for a substance and its intra-class correlation value suggests that the increased variability across assays for some substances is not a reflection of its binding activity. This analysis is affected to a great extent by the fact that so few assays were used within the same reference.

6.2.3 Variability in lnIC₅₀ and lnRBA Values For Selected Substances

Another approach for assessing the variability between substances is to evaluate the standard deviation of the lnRBA and lnIC₅₀ values of the 12 substances tested in at least nine of the 14 *in vitro* ER binding assays. These data are tabulated along with the corresponding median RBA values across assays in **Table 6-5**. The standard deviations were visually compared to determine which substances demonstrate more variability than others if the effects of assay and laboratory, which appear to be relatively small, are ignored. The overall variability presented in **Table 6-5** and the variability across and within assays shown in **Table 6-4** should be considered together.

The least amount of variation in binding affinity (based on assessing both lnRBA and lnIC₅₀ values) occurred for zearalenone, while the greatest variations (twice the lowest value) were observed for coumestrol, *o,p'*-DDT, and DES. Among the other substances, the variability in binding affinity was relatively similar among the different assays. Increased variability in the lnRBA and lnIC₅₀ values for coumestrol may be related to its much higher binding affinity for the purified proteins, especially ER α , compared to the cytosolic receptors (**Appendix D**).

Table 6-5 Variability in Standard Deviations for lnRBA and lnIC₅₀ Values For Selected Substances

Substance ^a (CASRN)	Median ^b RBA	# of Assays	lnRBA		lnIC ₅₀	
			Standard Deviation	N ^c	Standard Deviation	N ^c
4-Hydroxy- tamoxifen (68047-06-3)	168	13	1.36	18	1.68	10
DES (56-53-1)	127	14	2.01	38	3.20	26
Estrone (53-16-7)	45	13	1.49	18	1.57	8
Estriol (50-27-1)	15.8	12	1.36	16	0.89	6
Zearalenone (17924-92-4)	15.0	9	0.84	11	0.76	8
Tamoxifen (10540-29-1)	5.0	14	1.91	21	1.68	13
Coumestrol (479-13-0)	3.1	11	2.30	15	2.51	9
HPTE (2971-36-0)	1.45	10	1.15	12	1.14	10
Genistein (446-72-0)	1.30	11	1.74	18	1.64	12
<i>o,p'</i> -DDT (789-02-6)	0.038	10	2.27	15	1.87,	12
Bisphenol A (80-05-7)	0.031	14	1.63	22	1.54	15
Kepone (143-50-0)	0.027	9	1.37	11	1.07	8

^aSubstances that had been tested in at least 9 of the 14 *in vitro* ER binding assays; DES =diethylstilbestrol; *o,p'*-DDT=*o,p'*-dichlorodiphenyltrichloroethane; HPTE=(2,2-Bis(*p*-hydroxyphenyl)-1,1,1,-trichloroethane.

^bThe median RBA value across assays, based on positive test data.

^cN indicates the number of RBA or IC₅₀ values used in the analysis.

6.3 Qualitative Assessment of *In Vitro* ER Binding Assay Performance

A qualitative comparative assessment of assay performance considered the relative ability of the 14 *in vitro* ER binding assays to identify substances with relatively weak ER binding affinities and to obtain higher RBA values for the same set of substances. In conducting this assessment, it was assumed that all positive study results and all negative results for studies in which the highest dose tested was at least 100 µM were correct, for that assay. The 100 µM dose level criterion for negative studies was used to ensure that the protocol (in terms of test substance dose

levels) was minimally adequate for detecting weak positive responses. Thus, a positive assay reflects the intrinsic ability of the test substance to bind to the ER while a negative assay reflects difference in assay sensitivity rather than differences in the experimental protocol.

Due to the RUC assay having the largest database, this assay was used as the standard to compare with the performance of each of the 13 other *in vitro* ER binding assays. To conduct this assessment, the median RBA value was calculated for any substance tested positive in two or more tests using the same assay; otherwise the RBA value for a single positive test was used for that assay. Next, the resulting single or median RBA value for each substance in each assay was classified into one of seven RBA activity categories — 100, from <100 to 10, from <10 to 1, from <1 to 0.1, from <0.1 to 0.01, from <0.01 to 0.001, and <0.001. This classification scheme categorizes the range of RBA values into the seven orders of magnitude reported for ER binding substances (**Appendix D**). Substances that tested negative (i.e., no RBA value could be calculated) were classified as negative for that test. In situations where both positive and negative test results were obtained for the same substance using the same *in vitro* ER binding assay, the substance was classified as equivocal within the RBA value category for the positive assay(s). The RBA value category obtained for a substance tested in any *in vitro* ER binding assay other than the RUC assay was then compared and classified as higher, the same, lower, or negative in relation to the RBA value category obtained for that substance in the RUC assay. The results were then inspected to identify assays that appeared to have performed (1) better than, (2) as well as, or (3) not as well as the RUC assay. Improved performance for an assay would be demonstrated by a shift in the RBA values for substances tested in common to higher RBA value categories and to having fewer negative calls, compared to the RUC assay. Equal performance would be demonstrated by both the RUC and the assay being considered having the same RBA value categories for the majority of substances tested in common. Decreased performance for an assay would be demonstrated by a shift in the RBA values tested in common to lower RBA value categories and to having more negative calls, compared to the RUC assay. The results of this approach are summarized in **Table 6-6**.

This qualitative assessment is confounded by a number of limitations, including:

- The lack of multiple test data within an assay for the majority of the substances considered;

- The lack of a common set of substance to compare across all assays;
- The limited number of substances tested in common between the RUC and any other assay;
- The assumption that each test was conducted appropriately and that all test results were accurate for that assay;
- The arbitrariness of the RBA value categories and the possible adverse effect substances with RUC RBA values near the boundary between any two RBA value categories have on the assessment; and
- The inherent complexity added to an assessment when equivocal test substances (i.e., those with multiple, discordant test results) are classified as positive only.

Despite the limitations, the assessment suggests that:

- The hER , hER -FP, hER , and rER assays performed better than the RUC assay, as demonstrated by a shift among the substances tested toward higher category RBA values.
- The GST-ERdef assays, except for GST-rtERdef, did not perform as well as the RUC assay, as demonstrated by a shift among the substances tested toward lower category RBA values and more substances classified as negative. Many of the negative tests were for substances classified as equivocal in the RUC assay and tested only once in the GST-ERdef assays, potentially limiting the validity of this conclusion. The GST-rtERdef assay performed as well as the human and rat ER / assays.
- The MCF-7 cell assay did not perform as well as the RUC assay (increased numbers of substances with lower RBA value categories/negative results), while the MCF-7 cytosol assay performed about the same as the RUC assay.
- For the two other animal based test methods, the MUC assay performed better than and the RBC not as well as the RUC assay.

Table 6-6 Qualitative Assessment of the Ability of Different ER Binding Assays to Detect Substances with Different Relative Binding Affinities (RBA Values) Compared to the RUC Assay

Assay	Result	RBA Value Range								Totals
		≥100	<100-10	<10-1	<1-0.1	<0.1-0.01	<0.01-0.001	<0.001	Negative	
RUC (97)^a	+	6	17	6	13	16	8	3	0	
	+/-	0	0	0	1	1	4	7	0	
	-								15	
hERα (48)	Higher	-	2	1	4	7	3	2	2	21
	same	3	4	1	4	6	0	0	2	20
	lower	1	1	0	3	0	0	-	-	5
	negative	0	0	0	0	1	0	1	-	2
hERα-FP (24)	Higher	-	0	1	2	4	2	1	1	11
	same	1	1	1	1	2	0	0	2	8
	lower	1	1	0	0	1	0	-	-	3
	negative	0	0	0	0	0	1	1	-	2
hERβ (32)	Higher	-	2	1	4	7	3	2	2	19
	same	3	4	1	4	6	0	0	2	9
	lower	1	1	0	3	0	0	-	-	4
	negative	0	0	0	0	1	0	0	-	0
rERβ (24)	Higher	-	2	1	3	2	1	0	0	9
	same	3	4	1	2	0	0	0	2	12
	lower	0	0	0	0	0	0	-	-	0
	negative	0	0	0	0	1	0	1	-	2
GST- aERdef (28)	Higher	-	0	1	3	2	1	0	1	8
	same	3	5	1	1	1	0	0	1	12
	lower	0	0	0	1	0	0	-	-	1
	negative	0	0	0	0	1	2	4	-	7
GST- cERdef (27)	Higher	-	0	1	2	0	0	0	1	4
	same	3	5	1	2	3	1	0	1	16
	lower	0	0	0	0	1	0	-	-	1
	negative	0	0	0	0	0	2	4	-	6

Assay	Result	RBA Value Range								Totals
		≥100	<100-10	<10-1	<1-0.1	<0.1-0.01	<0.01-0.001	<0.001	Negative	
GST-hERαdef (28)	Higher	-	0	1	3	0	0	0	0	4
	same	2	4	0	2	1	0	0	2	11
	lower	1	1	1	0	2	0	-	-	5
	negative	0	0	0	0	1	3	4	-	8
GST-mERαdef (27)	Higher	-	0	1	2	0	0	0	0	3
	same	2	5	0	2	1	0	0	2	12
	lower	1	0	1	0	3	0	-	-	5
	negative	0	0	0	0	0	3	4	-	7
GST-rtERdef (29)	Higher	-	2	1	4	2	2	3	2	16
	same	3	3	0	1	2	0	0	1	10
	lower	0	0	1	0	0	0	-	-	1
	negative	0	0	0	0	0	1	1	-	2
MCF-7 cells (21)	Higher	-	0	0	0	0	1	0	2	3
	same	1	2	1	1	2	0	0	0	7
	lower	3	5	2	0	1	0	-	-	11
	negative	0	0	0	0	0	0	0	-	0
MCF-7 cytosol (31)	Higher	-	0	2	3	1	2	0	0	8
	same	4	10	3	0	1	0	0	0	18
	lower	0	2	0	1	1	0	-	-	4
	negative	0	0	0	0	0	1	0	-	1
MUC (24)	Higher	-	3	0	2	2	3	1	0	11
	same	1	1	1	1	4	0	0	1	9
	lower	1	0	0	2	0	0	-	-	3
	negative	0	0	0	0	1	0	0	-	1
RBC (22)	Higher	-	1	0	1	0	0	0	0	2
	same	3	3	2	0	0	1	0	0	9
	lower	1	0	1	0	3	0	-	-	7
	negative	0	0	0	0	1	3	2	-	4

^aNumber of substances.

Assessment based on substances tested in the RUC assay and at least one other *in vitro* ER binding assay. Data for the RUC assay entered as the number of positive (+), equivocal (+/-) (i.e., the substance was tested in more than one test with both positive and negative results obtained), and negative (-) calls for substances tested in that assay. Higher, the same, lower, and negative results signifies the occurrence of a higher, the same, lower, or negative RBA values compared to the corresponding RBA value obtained in the RUC assay for the same substance. Negative test method results in which the highest dose tested was <100 μ M were not included in this assessment.

6.4 Performance of *In Vitro* ER Binding Assays

The *in vitro* ER binding assays that are the most useful as a screen for endocrine disruptors are those that are the most sensitive (i.e., have the greatest ability to detect weak ER-binding substances) and the most reliable (i.e., exhibit the lowest variance) (see **Section 7**). In addition, it might be anticipated that those assays that use ER derived from the species of interest (e.g., human for predicting human-related effects, wildlife species for predicting effects in wildlife) might be the most informative. Finally, when taking animal welfare and human health and safety issues into consideration, assays that do not use ER obtained from experimental animals or ones that do not use radioactivity, respectively, might be of the greatest utility.

The results of the quantitative and qualitative assessments of the performance of the 14 *in vitro* ER binding assays evaluated in this BRD, as well as the results of an assessment of the utility (source of ER, absence of animal use, absence of the use of radioactivity) of the various assays, are summarized in **Table 6-7**. Based on these assessments, the hER_α, hER_α-FP, hER_β, and GST-rERdef assays appear to offer the greatest overall performance and utility as screening assays. The receptor used in the GST-rERdef assay is derived from the rainbow trout and thus might be less relevant for the screening of substances that might affect endocrine function in humans. However, this assay might have greater utility in screening for ED substances that might impact wildlife. The relative utility of ER_α versus ER_β assays in a screening paradigm needs further consideration. Among the substances tested in both the assays, 55% produced a higher RBA value in a hER_α assay, while 24% produced a higher RBA value in a hER_β assay. This suggests that a hER_α assay might perform better in a screening battery. As another consideration, the ER_α protein predominates in the uterus, while the ER_β protein is predominant in the prostate gland (Kuiper et al., 1997). Thus, inclusion of both types of estrogen receptors in a screening battery might be advantageous. However, among the 82 substances tested in common between the two assays, only two substances were discordant (i.e., one test substance was positive in a hER_α assay but negative in a hER_β assay, and vice-versa), suggesting that either assay would perform equally well in a screening battery.

Table 6-7 Summary of *In Vitro* ER Binding Assay Performance

Assay	Quantitative Performance ^a	Qualitative Performance ^b	Use of Experimental Animals ^c	ER from Species of Interest ^d	Non-radioactive Technology ^e
RUC	0				
hER	0	+	+	+	
hER -FP	0	+	+	+	+
hER	0	+	+	+	
rER	0	+	+		
GST-aERdef	0	-	+		
GST-cERdef	0	-	+		
GST-hER def	0	-	+	+	
GST-mER def	0	-	+		
GST-rtERdef	0	+	+	+	
MCF-7 cells	0	-	+		
MCF-7 cytosol	0	0	+		
MUC	0	+			
RBC	0	-			

^aThe quantitative assessment did not convincingly indicate that any single assay performed better than any other assay

^bThe RUC assay was used as the standard assay in the qualitative assessment; + = assays with improved performance; 0 = assays with similar performance; - = assays with lower performance than the RUC assay.

^cUtility (+) based on the lack of need for experimental animals.

^dUtility (+) based on the use of ER from a species of direct interest (i.e., human ER for human health, a wildlife species for ecological effects).

^eUtility (+) based on the use of non-radioactive technology.

6.5 General Strengths and Limitations of *In Vitro* ER Binding Assays

Competitive binding assays indicate whether a substance can interact with the target receptor by its ability to displace the natural ligand. These assays do not provide sufficient evidence to conclude that a substance is an agonist or an antagonist, or take into consideration other mechanisms of action that may lead to endocrine disruption (Zacharewski, 1998). However, *in vitro* binding assays can be important components of a battery of tests and are suitable for screening, because they:

- Are cost-effective;
- Are rapid and relatively easy to perform;

- Are based on a easily quantitated, well-elucidated mechanism of action (i.e., binding to a specific protein);
- Are sensitive (50 fmol ER/mg protein can be detected);
- Can be performed using small amounts of test substances;
- Can be used to test multiple substances simultaneously; and
- Can be easily standardized among laboratories.

These assays have limitations also, including:

- Inability to distinguish agonists from antagonists; and
- Potential generation of false positive and false negative results.

In terms of false positive results, the substance might disrupt the binding of the radioactive ligand to the ER by deactivating the receptor or decrease binding via noncompetitive inhibition (Kupfer, 1988). The latter might occur at high concentrations of the test substance. For false negative results, the accurate measurement of rapidly dissociating, low affinity ligands can be difficult because the bound ER and ligand are not in equilibrium when the unbound ligand is washed away from the receptor. Under these conditions, low affinity ligands are more likely to dissociate from the ER. This dissociation is a concern when the receptor or ligand is bound to a solid support such as charcoal that is used in traditional competitive ER binding assays (National Academy of Sciences, 1999). Assays that use FP to assess ER changes would not be affected by this concern. Other mechanisms for obtaining a false negative response include metabolic activation of the test substance to an active intermediate, which subsequently binds to the ER, incomplete solubility in the assay buffer, or incompatibility with assay conditions. Because traditional ER binding assays do not include the enzymes and co-factors required for metabolic activation, some potential ER binding substances will be missed. A possible solution to this limitation is to develop *in vitro* ER binding assays that include a metabolic activation system, as has been conducted in some ER TA assays (Charles et al., 2000; Sumida et al., 2001).

6.6 Conclusions and Recommendations

Although a large number of substances have been tested in *in vitro* ER binding assays, relatively few substances have been tested more than once in the same assay or in multiple assays.

Furthermore, as the primary focus of many of the investigations using *in vitro* ER binding assays has been at understanding mechanisms of binding and transcriptional activation and not at identifying substances with ER binding activity, much of the published data are of limited value in terms of an analysis of performance. Although these limitations weaken the validity of any assessment of *in vitro* ER binding assays, some general conclusions can be made.

The quantitative assessment of lnRBA and lnIC₅₀ values determined that the effect of substances on the variation in RBA and IC₅₀ values was much greater than the effect of assay type, and that significant differences in performance among the different *in vitro* ER binding assays were not present. One limitation of the quantitative assessment was that this approach does not consider situations in which a substance was classified as negative and positive in different tests using the same assay. The qualitative assessment considered whether RBA values (single or median) obtained for substances tested in each of 13 assays were within the same log range as the corresponding values obtained for the same substances in the RUC assay, and whether substances reported as positive or negative in the RUC assay were classified as negative or positive, respectively, in other assays. The RUC assay was selected as the assay for comparison because it had the largest database with respect to the number of substances tested and the number of laboratories using the procedure. The explicit assumption in this assessment was that an assay would perform as well as or better than the RUC assay if it demonstrated similar or higher RBA values and had the same or fewer negative calls for the same set of substances, respectively. Using this approach, the hER /hER -FP, hER /rER , GST-rERdef, and the MUC assays appear to have performed better than the RUC assay, while the MCF-7 cytosol assay appears to have performed about as well as the RUC assay. The remaining eight assays did not perform as well as the RUC assay but this may reflect the level of usage and the types of substances tested rather than a lack of performance. Similar to the quantitative assessment, this approach is limited by the lack of multiple test data within an assay for most of the substances considered, and by the lack of a common substance database to compare across all assays. The assessment also assumes that each test was conducted appropriately and that the test results were accurate.

Taking into account the available *in vitro* ER binding assay database and the various quantitative and qualitative assessments conducted on the 14 *in vitro* ER binding assays considered in this BRD, the following recommendations can be made in regard to the use of such assays as screening test methods within a battery of Tier 1 endocrine disruptor tests.

- Based on a consideration of such factors as relative performance, elimination of animal use, the use of the ER from the species of interest, and the use of alternatives to radioactive substances, the hER , hER -FP, and hER assays should have the highest priority for validation as screening assays for human health-related issues, while the GST-rtERdef assay might be preferred when screening for substances that pose a hazard to wildlife. Due to an inability to conduct an adequate assessment of assay reliability (see **Section 7**), reliability was not considered in making these recommendations. However, it might be expected that assays which use semi-purified or purified ER proteins would be more reliable than those based on extracts of ER from animal tissues.
- In conducting future validation studies with these assays, the RUC assay should be used as the reference test method. The RUC assay is currently undergoing validation efforts sponsored by the U.S. EPA and the resulting performance and reliability information could be used to establish minimal performance standards for other assays.
- Formal validation studies should be conducted using appropriate substances covering the range of expected RBA values to adequately demonstrate the performance characteristics of the *in vitro* ER binding assays recommended as possible screening assays. A list of potential test substances for use in such a validation effort is provided in **Section 12**.
- There is little information about the ER binding activity of metabolites of xenobiotics and it is not clear whether metabolic activation needs to be included in *in vitro* ER binding test methods used as screening assay. This issue should be considered prior to the implementation of future validation studies.

7.0 *IN VITRO* ER BINDING TEST METHOD RELIABILITY ASSESSMENT

7.1 Introduction

The ICCVAM Submission Guidelines (ICCVAM, 1999) request information about the assessment of test method reliability¹. This includes a rationale for selecting the substances used to evaluate intra- and inter-laboratory reproducibility, discussion of the extent to which the substances tested represent the range of possible test outcomes, and a statistical analysis of intra- and inter-laboratory reproducibility. In addition, measures of central tendency and variation for historical negative and positive control data and an assessment of the historical control variability should be conducted.

However, no formal validation studies to assess *in vitro* ER binding assay inter- and intra-laboratory reproducibility have been conducted, and the nature of the current database for these assays precludes a formal analysis. Historically, investigators have used these assays primarily to gain insight into the mechanisms of the binding of a ligand to the ER, to compare the relative binding of different ligands to ER isolated from different tissues and/or species, and to understand the process of ER-induced TA. Only relatively recently have ER studies been conducted to investigate the biological activities of putative endocrine disruptors.

Despite these limitations, a quantitative assessment of IC₅₀ and RBA values was conducted to assess the inter-laboratory reproducibility of each of the 14 *in vitro* ER binding assays considered in this BRD. The assessment was based on the 238 substances tested in at least two assays (**Appendix E**), and was limited to individual tests that resulted in an IC₅₀ or RBA value (i.e., the substance was classified as positive).

¹ Reliability is a measure of the degree to which a test can be performed reproducibly within and among laboratories over time, where reproducibility is the variability between single test results obtained in a single laboratory (intra-laboratory reproducibility) or in different laboratories (inter-laboratory reproducibility) using the same protocol.

7.2 Quantitative Assessments of Inter-laboratory Reproducibility

To reduce the extent of skewness in the data prior to conducting the quantitative assessments, the two outcome variables for *in vitro* ER binding assays — the RBA and the IC₅₀ values — were transformed using the natural log. Studies that did not result in an IC₅₀ and/or RBA value were excluded from consideration. Estimates of variance were compared across substances within the same assay, across substances without regard to the assay, and across assays without regard to the substances. A comparison of variances provides insight into which assays are the most reliable (i.e., all other aspects being equal, the smaller the variance, the more reliable the assay). Given the large number of data points for modeling, the general linear models (GLM) used in this analysis are robust, although some skewness may yet exist with the data. To simplify the comparison, each literature citation was considered an independent assessment (designated here as a ‘reference’).

As described in **Section 6**, two-way and three-way analysis of variance models were performed with random effects to estimate the intra-class correlation of substances. A high correlation value indicates that the lnRBA or lnIC₅₀ values are more similar within groups than among groups, where groups can be defined by assay or by reference. Estimates of variance for each model component and intra-class correlation are presented to show which factors (substance, assay, or reference) are responsible for the greatest variation in the lnRBA and lnIC₅₀ values. Due to limitations in the database with regard to the number of substances tested in multiple assays and to the number of independent tests performed for a substance using the same assay, the results of these analyses must be viewed with caution.

Initially, all data representing all substances, assays, and references were considered, and unique data (i.e., substances tested only in a single assay) were excluded from subsequent analyses. Information on the distribution of lnRBA and lnIC₅₀ values, as a function of datapoints, assays, and references are provided in **Section 6.2**. Consistent with the quantitative analysis on performance, the lnIC₅₀ and the lnRBA values for 17 β -estradiol were omitted from these analyses.

7.2.1 Measures of Intra-Class Correlation

The intra-class correlation, r_i , measures the percentage of variation in y , the outcome variable, explained by a given component or set of components. The model is $y = \text{substance} + \text{assay} + \text{reference}$. **Table 7-1** contains the components of variance for each variable adjusted for the other two variables (see also **Section 6**). Interpretation of this analysis is limited to factors that impact on reliability; factors that impact on assay performance are discussed in **Section 6**. From this analysis, it appears that the $\ln\text{RBA}$ or $\ln\text{IC}_{50}$ values calculated for a specific substance were generally consistent irrespective of how many times a substance was tested using the same assay.

Table 7-1 Components of Variance for Each Variable Adjusted for the Other Two Variables – Reliability Assessment

	Outcome, y (% variation)	
	$\ln\text{RBA}$	$\ln\text{IC}_{50}$
Var(substance)	8.34	8.49
Var(assay)	0.38	0.34
Var(reference)	1.40	2.01
Var(error)	1.75	2.44
Corr ($y_{ijk}, y_{ij'k}$)	0.70	0.64
Corr ($y_{ijk}, y_{ijk'}$)*	0.73	0.67
Corr ($y_{ijk}, y_{ij'k}$)	0.82	0.79

*A high correlation was found for a substance tested in the same assay (i.e., the variation in response of a substance within an assay was similar to that observed across assays).

The high correlation suggests that little variation existed in test results for an individual substance tested multiple times. However, because the majority of repeat tests were conducted on substances that were relatively potent in terms of *in vitro* ER binding, it is not known if similar variances would be found among weakly binding substances.

7.2.2 Evaluation of Substances Tested in Nine or More *In Vitro* ER Binding Assays

In this analysis, the variances for the RBA values of 12 substances that had been tested in at least 9 of the 14 *in vitro* ER binding assays were determined. The variances and sample sizes for these 12 substances are provided in **Table 7-2**, ranked in descending order according to the median RBA value based on all positive test data. Only assays for which

Table 7-2 Variance of lnRBA by Substance and Assay – Reliability Assessment^a

Substance ^b (CASRN)	Median ^c RBA	#of Obs/ # Assays	hER α ^d	hER α -FP ^d	hER β ^d	MCF-7 cytosol ^d	MUC ^d	RUC ^d	p1*	p2**
4-Hydroxy- tamoxifen (68047-06-3)	168	18/13	0.28 (3)	1.82 (3)					0.08	0.15
DES (56-53-1)	127	38/14	0.99 (3)	0.45 (4)			0.60 (7)	3.62 (11)	0.15	0.99
Estrone (53-16-7)	45	18/13				2.40 (3)		0.98 (4)	0.73	na ^e
Estriol (50-27-1)	15.8	16/12				2.42 (4)			0.53	0.64
Zearalenone (17924-92-4)	15.0	11/9	All n \leq 2						0.42	na
Tamoxifen (10540-29-1)	5.0	21/14	0.44 (3)					2.95 (4)	0.02	0.10
Coumestrol (479-13-0)	3.1	15/11	0.79 (3)						0.02	0.25
HPTE (2971-36-0)	1.45	12/10						1.53 (3)	0.82	na
Genistein (446-72-0)	1.30	18/11	1.07 (4)		0.97 (3)				0.11	0.18
Bisphenol A (80-05-7)	0.031	22/14	1.36 (3)					1.25 (5)	1.25	0.60
<i>o,p'</i> -DDT (789-02-6)	0.038	17/12						2.97 (5)		
Kepone (143-50-0)	0.027	11/9						1.39 (3)	0.60	na

^aOnly assays where a variance could be calculated for at least one of the 12 substances are listed. The variance for a particular assay could be calculated only if a particular substance was tested three or more times in that assay; empty cells indicate insufficient data to calculate a variance. The p values could be calculated only if there were two observations from at least three or more assays; a missing p-value indicates insufficient data.

^bSubstances that had been tested in at least nine of the 14 *in vitro* ER binding assays; DES = diethylstilbestrol; *o,p'*-DDT = *o,p'*-dichlorodiphenyltrichloroethane; HPTE = (2,2-Bis(*p*-hydroxyphenyl)-1,1,1,-trichloroethane

^cThe median RBA value across assays, based on positive test data.

^dThe numbers in parenthesis indicate the numbers of replicate tests.

^ena = No p value could be calculated since there was either no values or only one value per assay x response combination.

*p1 tests whether there is a significant difference among all assays used; unadjusted for references.

**p2 tests whether there is a significant difference among all assays used; adjusted for references.

could be calculated are included, and most of these variances were based on three or four values only. Due to the lack of sufficient data, a corresponding analysis of IC_{50} values was not conducted.

The similarity between the p_1 and p_2 values for most of these substances suggests that there were no significant differences in the performance of the assays by different laboratories (a measure of assay reliability). However, DES and coumestrol exhibited considerably less variability when the analysis was adjusted for the reference (i.e., p_2 is much greater than p_1), suggesting that laboratory-specific differences in testing of this substance were responsible.

7.2.3 Variability in Standard Deviation for $\ln RBA$ and $\ln IC_{50}$ Values by *In Vitro* ER Binding Assay

Because of insufficient data on substances tested in the same assay within or across laboratories, separate correlations between pairs of the 14 assays were not calculated. However, standard deviations of the mean of the $\ln RBA$ and the $\ln IC_{50}$ values were inspected to see which assays have the least or the most variability in their responses (**Table 7-3**). A major limitation of this analysis is that the same substances were not tested in each assay. An additional limitation is the varied number of substances tested more than once in each assay. These limitations will affect any interpretation of the results. The assays in **Table 7-3** are sorted in descending order based on the number of different substances tested in each assay.

Not unexpectedly, for the same set of substances, there appears to be more variability in the IC_{50} values than in the corresponding normalized RBA values. The standard deviations for the majority of substances clustered around a median of 3.23 for the $\ln RBA$ values and 3.52 for the $\ln IC_{50}$ values. The standard deviations for the $\ln RBA$ values vary from a low of 2.92 for the rER assay to a high of 5.09 for the RBC assay, while the corresponding standard deviations for the $\ln IC_{50}$ values vary from a low of 2.95 for the GST-hER assay to a high of 4.85 for the RBC assay. The MCF-7 cells, hER, GST-hER and MCF-7 cytosol assays exhibited similar and relatively lower standard deviations for $\ln RBA$ values, while the GST-aERdef, hER and GST-rtERdef assays exhibited similar and relatively lower standard deviations for $\ln IC_{50}$ values. The

RBC assay exhibited the largest standard deviation for both the $\ln\text{RBA}$ and $\ln\text{IC}_{50}$ values. Based on this analysis, the hER assay appears to be the most reliable, while the RBC assay appears to be the least reliable. However, these conclusions must take into account the number of substances that have been tested in each ER assay and, although not specified, the number of laboratories that generated the data. In general, the standard deviation increases as the number of substances tested in an assay increases or as more laboratories are involved.

Table 7-3 Standard Deviation for $\ln\text{RBA}$ and $\ln\text{IC}_{50}$ Values for *In Vitro* ER Binding Assays

Assay	Number of Different Substances	$\ln\text{RBA}$		$\ln\text{IC}_{50}$	
		Standard Deviation	n ^a	Standard Deviation	n ^a
RUC	100	4.34	164	4.30	90
hER	87	3.26	112	3.69	24
hER	74	3.03	91	3.24	30
MCF-7 cytosol	63	3.07	72	3.55	15
MCF-7 cells	58	2.94	49	3.46	2
GST-rtERdef	43	3.20	43	3.26	43
MUC	33	3.49	49	3.37	35
GST-hER def	29	3.01	29	2.95	29
rER	28	2.92	28	-	0
GST-aERdef	25	3.19	25	3.15	25
GST-cERdef	21	3.68	21	3.68	21
RBC	21	5.09	22	4.85	8
GST-mER def	19	3.53	19	3.52	19
hER -FP	19	3.84	28	3.80	28

^aTotal number of data points considered in the analysis.

7.2.4 Variability in the IC_{50} for 17β -Estradiol

The most extensive database within and across assays is for 17β -estradiol, the natural estrogen commonly used as the reference substance in *in vitro* ER binding assays for calculating the RBA value of a test substance. However, because the RBA value for this substance is arbitrarily set at 100, this measure of binding cannot be analyzed for variability. In contrast, an analysis of the IC_{50} values of 17β -estradiol, where reported, provides a means for assessing assay reproducibility. Fifty-eight IC_{50} values were available for 17β -estradiol in the ER binding database (**Appendix D**). The variability in the natural log of IC_{50} values of 17β -estradiol was

compared across assays. As the sample size within each assay is quite small, only descriptive statistics of this parameter are presented (**Table 7-4**). The IC₅₀ values are sorted in descending order based on the number of times 17 β -estradiol was tested in each assay.

Table 7-4 Standard Deviation for IC₅₀ Values Obtained for 17 β -Estradiol

Assay	N ^a	Standard Deviation ^b
RUC	13	0.90
MUC	10	2.21
hER	9	0.99
hER -FP	7	0.61
hER	4	0.78
MCF-7 cytosol	3	4.06
GST-hER def	3	0.44
GST-rtERdef	2	0.044
GST-aERdef	2	0.15
GST-cERdef	1	
GST-mER def	1	
MCF- 7 cells	1	
RBC	1	
rER	1	

^aNumber of data points considered.

^bStandard deviations could not be calculated for single test data.

In this analysis, the greater the standard deviation, the less reliable is the assay. Since the IC₅₀ values of 17 β -estradiol for the rER, RBC, MCF-7 cells, GST-mER def, and GST-cERdef assays were reported by one laboratory only, no standard deviations could be calculated. Although the standard deviations for the IC₅₀ values were very small for the GST-aERdef, GST-rtERdef, and GST-hER def assays, only two or three data points were reported for these assays. Among the assays with at least six data points (hER -FP, MUC, RUC, hER), the standard deviations in the lnIC₅₀ values are generally similar except for the hER -FP assay where the value is smaller. Although this decreased standard deviation suggests that the hER -FP assay is the most reliable of these four assays, the hER -FP assay data were generated by fewer laboratories, which may have impacted on the extent of variability.

7.3 Reliability of *In Vitro* ER Binding Assays

The *in vitro* ER binding assays that are the most useful as a screen for endocrine disruptors are those that are the most sensitive (i.e., have the greatest ability to detect weak ER-binding substances) (see **Section 6**) and the most reliable (i.e., exhibit the lowest variance). The results of the quantitative assessments of the comparative reliability of the 14 *in vitro* ER binding assays evaluated in this BRD are summarized in **Table 7-5**.

Table 7-5 Summary of *In Vitro* ER Binding Assay Reliability

Assay	lnRBA ^a	lnIC ₅₀ ^a	IC ₅₀ 17 β -Estradiol ^c
RUC	-	-	0
GST-aERdef	0	+	?
GST-cERdef	-	0	?
GST-hER def	+	+	?
GST-mER def	0	0	?
GST-rtERdef	0	+	?
hER	0	0	0
hER -FP	-	-	+
hER	+	+	?
MCF-7 cells	+	0	?
MCF-7 cytosol	+	0	?
MUC	0	+	0
RBC	-	-	?
rER	+	?	?

^a Reliability based on standard error term for lnRBA values (**Table 7-3**); more reliable = +; average reliability = 0; less reliable = -.

^a Reliability based on standard error term for lnIC₅₀ values (**Table 7-3**); more reliable = +; average reliability = 0; less reliable = -; ? = the number of observations was too small to make a determination.

^c Reliability based on variance analysis of lnIC₅₀ values for 17 β -estradiol (**Table 7-4**); most reliable = +, average reliability = 0; least reliable = -; ? = the number of observations was too small to make a useful determination.

Based on a weight-of-evidence approach, the GST-hER def and hER assays appear to offer the greatest overall reliability (both assays had two reliable categories). However, due to the absence of formal validation studies to assess reliability and to the paucity of the data on which this reliability assessment is made, the decision to select any one assay or group of assays over another appears to be arbitrary.

7.4 Conclusions and Recommendations

Although a large number of substances have been tested in *in vitro* ER binding assays, relatively few substances have been tested more than once in the same assay or in multiple assays, and no formal validation studies have been conducted to assess reliability. A quantitative assessment was conducted using the available IC_{50} and RBA data after being log-normal transformed to reduce possible skewness. One limitation of this approach was that situations in which a substance was classified as negative and positive in different tests using the same assay was not considered.

An analysis of the variances for the RBA values of 12 substances that had been tested in at least nine of the 14 *in vitro* ER binding assays suggested that there were no significant differences in the reliability of the assays as performed by different laboratories. Inspection of the standard errors of the mean of the $\ln RBA$ and the $\ln IC_{50}$ values suggested that the RUC assay appeared to be the most reliable, while the RBC assay appeared to be the least reliable. A major limitation of this analysis is that the same substances were not tested in each assay and that the number of substances that have been tested in each ER assay or the number of laboratories that generated the data was not considered.

A comparison of the variability in $\ln RBA$ and $\ln IC_{50}$ values across assays, ignoring substance effects, indicated that the GST-hER_{def} and hER_{def} assays were the most consistent and the RBC assay was the least consistent among the 14 assays evaluated. An analysis of the variability in the $\ln IC_{50}$ for 17 β -estradiol, the reference estrogen for these assays, indicated that the most consistent results were obtained with the hER_{def}-FP assay, while the MUC, RUC, and hER_{def} assays exhibited somewhat greater but comparable variances. The low variability associated with the hER_{def}-FP assay, however, might be a reflection of the small number of laboratories that have reported RBA values using this method. Data were too limited to evaluate the other *in vitro* ER binding assays.

Taking into account the available *in vitro* ER binding assay database and the various quantitative assessments conducted on the 14 *in vitro* ER binding assays considered in this BRD, the

following recommendation can be made in regard to the use of such assays as screening test methods within a battery of Tier 1 endocrine disruptor tests.

- Despite inferences that the GST-hER def and hER assays appear to be the most reliable among the 14 *in vitro* ER binding assays considered in this BRD, an adequate assessment of assay reliability cannot be performed based on the limited database available. However, it might be expected that assays that use semi-purified or purified ER proteins would be more reliable than those based on extracts of ER from animal tissues.
- It is essential that validation studies be conducted to assess assay reliability and that these validation studies use appropriate substances covering the range of expected RBA values. A list of potential test substances for use in such a validation effort is provided in **Section 12**.

8.0 QUALITY OF DATA REVIEWED

8.1 Extent of Adherence to GLP Guidelines

Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with GLP guidelines, which are nationally and internationally recognized rules designed to produce high-quality laboratory records. GLPs provide a standardized approach to report and archive laboratory data and records, and information about the test protocol, to ensure the integrity, reliability, and accountability of a study (U.S. EPA, 1994a,b; FDA, 1994).

Based on the available information, none of the published *in vitro* ER binding studies identified for this BRD appear to have been conducted in compliance with national or international GLP guidelines.

8.2 Assessment of Data Quality

Formal assessments of data quality, such as a quality assurance (QA) audit, generally involve a systematic and critical comparison of the data provided in a study report or published paper to the laboratory records generated for a study. No attempt was made to formally assess the quality of the *in vitro* ER binding data included in this BRD. The published data on the competitive binding of substances to the ER were limited to RBA and, to a lesser extent, IC₅₀ and K_i values. Auditing these reported values would require obtaining the original data for each ER binding experiment, which is not readily available.

An informal assessment of the ER binding publications revealed limitations that complicate interpretation of the *in vitro* ER binding assay data (**Appendix D**):

- *Insufficient methods information*: A relatively large number of publications contained limited details about the methods used to conduct the studies. In some cases, publications reported that the methods were “performed as previously described,” and in many of these cases the cited publication referenced another publication for experimental details. Following this trail of references made it difficult to determine the actual protocol used to produce the data reported in the publication being abstracted.
- *Inconsistent nomenclature of test substances*: Most publications did not provide CASRNs for the substances tested, which in some cases made an unequivocal identification difficult. For

example, 19 publications reported results for a hydroxylated form of tamoxifen. Most laboratories reported testing “4-hydroxytamoxifen;” however, a few publications used less specific substance names, such as “monohydroxytamoxifen” and “hydroxytamoxifen,” which do not specify the location of the hydroxy group on the parent molecule. As a result, it is not possible to conclude definitively that these three names referred to same substance.

- *Data reporting:* A few publications calculated the RBA value of a test substance using the IC₅₀ value of 17 β -estradiol reported in another publication. Thus, it could not be determined whether the test substance and 17 β -estradiol were evaluated concurrently in the same experiment. Additionally, much of the data reported in the publications were RBA values only, with no accompanying error term provided to assess the quality of the estimate. Thus, the variability of the experimental data could not be assessed.
- *High number of unreplicated experiments:* The majority of the substances tested in the ER binding experiments have not been tested by other laboratories, and thus, the results are unconfirmed. Of the 638 substances included in this BRD, 376 (59%) were tested by one laboratory only.
- *Graphical presentation of data:* Some publications presented the results of ER binding experiments in graphical format only. A majority of these publications presented IC_x data in a semi-log plot (e.g., % [³H]17 β -estradiol vs. log concentration of competitor). In these cases, IC₅₀ values were estimated from the graphs, and used to calculate the corresponding RBA values. These estimations might contribute to some of the variability seen in the RBA values.

8.3 Quality Control Audit

NICEATM staff conducted a quality control (QC) audit of the ER binding database provided in **Appendix D**. In conducting this audit, data input into the database was checked against the original sources and corrected if an entry error had been made.

8.4 Need for Data Quality

Data quality is a critical component of the test method validation process. To ensure data quality, ICCVAM recommends that all of the data supporting validation of a test method be available with the detailed protocols under which the data were produced. Original data should

be available for examination, as should supporting documentation, such as laboratory notebooks. Ideally, the data should adhere to national or international GLP guidelines (ICCVAM, 1997).

All of the *in vitro* ER binding assay data included in this BRD were obtained from peer-reviewed scientific articles reporting the results of studies conducted at facilities that do not typically perform studies in compliance with GLP guidelines. It should be noted that a majority of these studies were performed in response to basic research questions and/or to evaluate the binding affinities of estrogen analogs or new drugs, not to support pre-validation or validation of the test method, or the formal submission of data to regulatory agencies. Because these studies span three decades and a multitude of laboratories, verifying the integrity of the data via a formal audit process was not possible.

An informal assessment of the *in vitro* ER binding assay data showed that the test substances and data were not consistently represented in the same format. In addition, the methods were presented in varying levels of detail and completeness. Since the published data were not verified for their accuracy against the original experimental data, caution must be exercised when interpreting the quantitative and qualitative analyses performed in **Section 6**.

An important step towards acceptance of *in vitro* ER binding assay methods into a regulatory screening program is production of high quality data. To achieve this goal, it is recommended that any future pre-validation and validation studies on *in vitro* ER binding assays be conducted with coded substances and in compliance with national and international GLP guidelines. Ideally, the substances should be obtained from a common source, and distributed from a central location. Laboratories not able to perform studies in compliance with GLP guidelines should perform studies in the spirit of GLP. At a minimum, this would require detailed, accurate documentation of laboratory protocols, experiment-related notes, and data entries.

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9.0 OTHER SCIENTIFIC REPORTS AND REVIEWS

9.1 Availability of Other *In Vitro* ER Binding Data

A number of the peer-reviewed publications identified during the initial literature search that contained ER binding data were not abstracted for inclusion in this BRD. These include:

- Studies lacking either appropriate quantitative data (i.e., RBA or IC₅₀ values) or the necessary information to calculate IC₅₀ values;
- Studies for which test substances were not adequately identified;
- Studies containing data from unique procedures (e.g., T47D cells, bovine uterine cytosol); and
- Publications reporting results for only a few substances that had not been tested by any other investigator.

Recognizing that unpublished ER binding data may be available, a formal request was published in the *Federal Register* (Vol. 66, No. 57, pp.16278 – 16279) for data and/or information from completed studies using or evaluating ER binding assays. No information was received in response to this request.

It is known that some companies involved in the development of pharmaceuticals routinely use *in vitro* ER binding assays to screen substances for their potential estrogenic activity. However, these data are unpublished and have not been provided to NICEATM for consideration.

The U.S. EPA has a contract with Battelle Pacific Northwest National Laboratory (Richland, WA) to generate *in vitro* ER binding data to evaluate two QSAR ER binding models developed by scientists at the U.S. Food and Drug Administration National Center for Toxicological Research (FDA NCTR) and by Dr. Ovanes Mekenyan (Mekenyan et al., 2000). Initially, Battelle will test 25 substances in an *in vitro* ER binding RUC assay. The ultimate goal is to test a total of 300 substances for evaluation in the two QSAR models, which the U.S. EPA plans to use for priority setting of substances for the U.S. EPA EDSP. In addition, the American Chemistry Council (ACC) is sponsoring *in vitro* ER binding studies, using the RUC assay, at two laboratories that will be testing approximately 25 substances. Neither the U.S. EPA nor the ACC test results are available at this time.

While every effort was made to include all available, pertinent *in vitro* ER binding assay data in this BRD, some data may have been excluded inadvertently.

9.2 Conclusions of Other Scientific Reviews of *In Vitro* ER Binding Methods

To date, no independent peer reviews of *in vitro* ER binding assays have been conducted. However, two workshops addressed the use of these assays as potential endocrine disruptor screening methods. Although the strengths and limitations of these assays were discussed at both workshops, no effort was made to evaluate the reliability or performance of these assays. Some of the conclusions from these workshops are summarized below.

9.2.1 1996 Endocrine Disruptor Screening Methods Workshop

In vitro ER binding assays were discussed extensively at an Endocrine Disruptor Screening Methods Workshop held in July 1996 at Duke University in Durham, North Carolina. Gray et al. (1997) edited the proceedings of this workshop, which was co-sponsored by the U.S. EPA, the Chemical Manufacturer's Association (CMA), and the World Wildlife Fund (WWF).

The major strengths of *in vitro* cytosolic ER binding assays cited by the authors include:

- Sensitivity (can detect ER binding with as low as 50 fmol ER/mg protein);
- Specificity of response;
- Relatively short duration of the test;
- Fairly inexpensive;
- Well-documented; and
- Can be standardized.

The major limitations cited by the authors include:

- Do not distinguish between estrogen agonists and antagonists;
- Substances requiring metabolic activation would produce false negative results;
- Insolubility of test substance in assay buffer could produce a false negative result; and
- Denaturation effects of a test substance could produce false positive results.

In addition, the authors briefly discussed the major advantages and disadvantages of cell-free and whole-cell binding assays using hER. The major strength of these assays is their potential relevance to humans, while their major limitation is that they are relatively new methods with little published data.

9.2.2 1997 Workshop on Screening Methods for Detecting Potential (Anti-) Estrogenic/Androgenic Chemicals in Wildlife

In March 1997, the U.S. EPA, the CMA, and the WWF co-sponsored a workshop in Kansas City, Missouri that addressed the use of ER binding assays as screening methods for detecting potential (anti-) estrogenic chemicals in wildlife. Proceedings of this workshop were published by Ankley et al. (1998).

The major advantages cited by the authors of using ER binding assays as endocrine disruptor screens for wildlife include:

- Widespread acceptance and use; and
- Can be conducted with ER from various mammalian and non-mammalian species, including fish, reptiles and birds.

The major disadvantages include:

- Do not distinguish between agonists and antagonists; and
- Uncertainties regarding extrapolation across species.

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10.0 ANIMAL WELFARE CONSIDERATIONS

10.1 Refinement, Reduction, and Replacement Considerations

ICCVAM promotes the scientific validation and regulatory acceptance of new methods that refine, reduce, or replace animal use where scientifically feasible (ICCVAM, 1997; P.L. 106-545). Refinement, Reduction, and Replacement are known as the three Rs of animal protection. These principles of humane treatment of laboratory animals are described as:

- Refining experimental procedures such that animal suffering is minimized;
- Reducing animal use through improved science and experimental design; and
- Replacing animal models with non-animal procedures (e.g., *in vitro* technologies), where possible.

Combes (2000) and Phillips (2000) recommended that adequate consideration be given to animal welfare concerns by careful development and validation of all proposed endocrine disruptor screening methods. With respect to the proposed use of *in vitro* ER binding assays as screening methods to detect substances that potentially exhibit estrogenic or anti-estrogenic activity, it is important to evaluate the current level of animal use in these assays and to consider what opportunities exist for refining, reducing, or replacing procedures that use animals.

10.2 Use of Animals in *In Vitro* ER Binding Assays

Of the 14 *in vitro* ER binding assays considered in this BRD, three assays (RUC, MUC, RBC) require the collection of uterine tissue from female rats, mice, or rabbits. Because the animals are not treated with a test substance, treatment-related pain and suffering are avoided. Some investigators that use the RUC and MUC assays obtain the uterus from ovariectomized mature female rats, while other investigators use non-ovariectomized, sexually immature female rats. Some investigators prefer the former procedure because removal of the ovaries appears to increase uterine ER production in the rat for about five to 14 days after an ovariectomy. Thus, more ER can be obtained per gram of uterine tissue in comparison to the procedure using non-ovariectomized, sexually immature females. One investigator who uses uteri from ovariectomized rats in the RUC assay estimates that one average-sized mature rat uterus (~200 mg) generates enough cytosol to test one substance at six concentrations in triplicate (personal

communication, Dr. Hong Fang, NCTR). Corresponding information on the amount of cytosol generated from sexually immature rats was not obtained.

With respect to refining the uterine cytosol assays, procedures that are the least invasive and distressful to the animals should be used. As for reducing the number of animals used in these assays, protocols should maximize the number of substances that can be tested per gram of tissue, for example, by optimizing the protocol to use the lowest possible concentration of ER per assay tube. In addition, the use of sexually mature versus immature animals should be carefully considered. While the use of immature animals only would reduce the need for ovariectomies, using sexually immature animals, which have substantially smaller uteri than mature ovariectomized animals (e.g., 30-50 mg versus 200 mg for the rat), would require that more animals be used.

The other 11 *in vitro* ER binding assays considered in this BRD do not entail animal use. Two of these assays – the MCF-7 cell and MCF-7 cytosol assays – use a human cell line while the remaining assays use purified or semi-purified human or animal receptors derived from cDNA or ER-glutathione fusion proteins. The experimental systems using purified receptors, semi-purified receptors, or glutathione fusion proteins can be carried out in multi-well plates, which permit smaller reaction volumes and allow data collection to be partially or fully automated. With the potential for automation, these systems would be more economical to perform than the uterine cytosol assays, which require animal care and surgical costs. Another advantage to using purified (cloned) ER is that ER α and ER β can be used selectively.

The assays using human ER or the ligand binding domain of the human ER are directly relevant to humans, as compared to ER derived from rodent or rabbit tissues. However, because of the relative newness of these assays, they have not been used as extensively as the uterine cytosol assays for the routine testing of substances; thus, their reliability and performance have not been demonstrated to the same extent. Despite the lack of a substantial database on assays using purified and semi-purified ERs, these assays, with further development and validation, could potentially replace the use of uterine cytosol to determine the ER binding of substances.

11.0 PRACTICAL CONSIDERATIONS

11.1 Test Method Transferability

Test method transferability addresses the ability of a method to be accurately and reliably performed by multiple laboratories (ICCVAM, 1997). This definition includes laboratories experienced in the particular type of procedure, and otherwise competent laboratories with less or no experience in the particular procedure. It also addresses whether the necessary facilities, equipment, and trained staff to perform the method can be readily obtained, and whether the cost of the assay and the level of expertise or training needed are considered reasonable. The degree of transferability of a test method affects its inter-laboratory reproducibility.

The ICCVAM Submission Guidelines (ICCVAM, 1999) request a discussion of test method transferability with respect to the following factors:

- Availability of the facilities and the major fixed equipment needed to perform the test method;
- The training requirements for laboratory personnel to demonstrate proficiency with the test method;
- Costs involved in conducting the test; and
- Time needed to conduct the test.

11.1.1 Facilities and Major Fixed Equipment

The facilities needed to conduct ER binding assays are widely available, and the necessary equipment is readily available from major suppliers. Specific needs as related to the various *in vitro* ER binding procedures are described below. To ensure personnel and community safety, pertinent State or Federal regulations for the handling of hazardous and radioactive substances/wastes must be strictly adhered to.

Uterine Cytosol ER Binding Assays

Facilities: Standard toxicology, biochemistry, or molecular biology laboratory supplies, and an animal facility containing temperature, humidity, and light controls. A small animal surgical facility is recommended for laboratories that prefer to not purchase ovariectomized animals from animal suppliers.

Major Fixed Equipment: Refrigerated centrifuge, ultracentrifuge, and liquid scintillation counter.

MCF-7 Cells/Cytosolic Assays and Semi-Purified ER and ER or GST Fusion Proteins

Facilities: Standard cellular or molecular biology laboratory with cell culture capabilities.

Major Fixed Equipment: Liquid scintillation counter.

Purified Human ER Measured by Fluorescent Polarization

Facilities: Standard cellular or molecular biology laboratory.

Major Fixed Equipment: Fluorescence polarization instrument.

11.2 Training Considerations

Uterine Cytosol ER Binding Assays

Basic laboratory skills and training in small animal handling and surgery.

MCF-7 Cells/Cytosolic Assays

Basic laboratory skills and training in cell culture techniques.

Semi-purified ER and ER

Basic laboratory skills with training in molecular biology particularly cloning, cell culture techniques and protein purification.

GST Fusion Proteins

Basic laboratory skills with training in molecular biology particularly cloning, bacterial cell culture techniques and protein purification.

Purified Human ER Measured by Fluorescent Polarization

Basic laboratory skills.

11.3 Cost and Time Considerations

Table 11-1 provides information on the estimated cost per sample, the expected duration of the study, special equipment needed, and other considerations. The cost information provided was obtained from scientists working at not-for-profit institutions and would be an underestimate for studies conducted at contract laboratories in compliance with GLP guidelines. Where estimated costs are not provided, it is probably safe to assume that the costs for all of the uterine cytosol assays (RUC, MUC, RBA) are roughly equivalent. Similarly, it would be expected that the costs for the assays using semi purified ER or GST constructs and the cell culture assays would be roughly equivalent.

Table 11-1 Comparison of Costs, Time, and Special Equipment Needs of Different ER Binding Assays

Assay	Cost/ Test substance	Duration (hours)	Special Equipment	Other Considerations
RUC	\$135	~24 – 48	Liquid scintillation counter (\$15K - \$30K)	
MUC	n.a.	~24 – 48	Liquid scintillation counter (\$15K - \$30K)	
RBC	n.a.	~24 – 48	Liquid scintillation counter (\$15K - \$30K)	
MCF-7 cytosol	n.a.	~24 – 48	Liquid scintillation counter (\$15K - \$30K)	
hERα	n.a.	~24	Liquid scintillation counter (\$15K - \$30K)	
hERα-FP	\$65	~4	Fluorescence polarimeter (\$20K - \$35K)	No radioactive wastes. Proprietary fluorescein- labeled estrogen ligand.
hERβ	n.a.	~24	Liquid scintillation counter (\$15K - \$30K)	
rERβ	n.a.	~24	Liquid scintillation counter (\$15K - \$30K)	
GST-hERαdef	\$30	~8	Liquid scintillation counter (\$15K - \$30K)	
GST-mERαdef	\$30	~8	Liquid scintillation counter (\$15K - \$30K)	
GST-aERdef	\$30	~8	Liquid scintillation counter (\$15K - \$30K)	
GST-cERdef	\$30	~8	Liquid scintillation counter (\$15K - \$30K)	
GST-rtERdef	\$30	~8	Liquid scintillation counter (\$15K - \$30K)	
MCF-7 cells	n.a.	~24 – 48	Liquid scintillation counter (\$15K - \$30K)	

n.a. = Cost estimate not available in the literature or from laboratories conducting the assay.

12.0 MINIMUM PROCEDURAL STANDARDS FOR *IN VITRO* ER BINDING ASSAYS AND RECOMMENDATION OF SUBSTANCES FOR USE IN VALIDATION STUDIES

12.1 Introduction

Although published studies on the ability of substances to bind *in vitro* to the ER are relatively numerous, there are no published standard test guidelines for conducting such studies, and no formal validation studies have been performed to assess the reliability or performance of ER binding assays. To support the further standardization and validation of *in vitro* ER binding assays, minimum procedural standards for such assays and a recommended list of test substances for use in validation studies are provided. The minimal procedural standards and recommended test substances are based on a comparative evaluation of the 14 *in vitro* ER binding assays summarized and evaluated in this BRD (**Sections 6 and 7**). The RUC assay, which has been the most widely used method for identifying substances with ER binding activity, is proposed as the standard against which new tests should be evaluated.

12.2 Minimum Procedural Standards

12.2.1 Animal Studies

All studies utilizing animals should be approved by the Institutional Animal Care and Use Committee (IACUC) or its equivalent.

12.2.2 Dissociation Constant (K_d) of the Reference Estrogen

Irrespective of the source of the ER used, the dissociation constant, K_d , of the reference estrogen (e.g., 17 β -estradiol) must be determined each time the assay is performed. The purpose of determining K_d is to demonstrate that the assay system is valid (e.g., a finite number of high affinity receptors are saturated with ligand) and to optimize the system with respect to receptor and ligand concentration. The K_d is determined in a saturation binding experiment that involves adding increasing concentrations of the radiolabeled reference estrogen to the ER preparation and measuring binding to the ER (Motulsky, 1995). To calculate specific binding of the radiolabeled reference estrogen to the ER, nonspecific binding is measured at each radioligand concentration by the addition of a nonlabeled estrogen at a concentration that occupies all available receptors. The nonspecific binding is then subtracted from the total binding (in the

absence of nonlabeled compound) of the radiolabeled reference estrogen (Motulsky, 1995). The K_d of the reference estrogen, which reflects its affinity for the specific ER preparation, can then be calculated, and is used to determine the appropriate concentration of reference estrogen to be used in competitive binding assays. To determine the K_d , the ER must be exposed to the reference estrogen at concentrations spanning five to six orders of magnitude.

12.2.3 Preparation of Test Substances

Test substances must be dissolved in water or in a solvent that is miscible with water. For substances not sufficiently water soluble, absolute ethanol, or DMSO are proposed as solvents. Preference is given to ethanol since this solvent has been used in most of the studies conducted to date. Other solvents may be used as long as it can be demonstrated that they do not interact with the test system. A solvent control set of assay tubes must be included in each assay. It might be necessary to characterize the solubility of the test substance in several solvents to identify the optimal solvent to use in the ER binding assay.

12.2.4 Concentration Range of Test Substances

To minimize effort and costs in screening/testing, and in recognition that adding excessive amounts of a test substance can perturb the test system through physico-chemical mechanisms, most testing schemes include a limit dose (i.e., the highest dose that should be tested in the absence of solubility constraints). An agreed upon limit dose for *in vitro* ER binding screening assays has not been established. Historically, the highest dose tested in such assays has ranged generally from 1 to 100 μ M, with some tests conducted at doses as high as 1 mM. The IC_{50} values (and thus the RBA values) reported for substances tested in various *in vitro* ER binding assays cover six orders of magnitude below the IC_{50} for 17 β -estradiol, the reference estrogen. In the RUC assay, the median IC_{50} for 17 β -estradiol is 3.8 nM. Thus, if testing for ER binding substances requires the ability to detect substances with an IC_{50} that is at least six orders of magnitude lower than that of 17 β -estradiol, then the limit dose (unless precluded by chemical properties such as solubility) should be above 4 mM (e.g., 10 mM) to allow for the detection of an IC_{50} in the concentration range of interest. However, if five orders of magnitude are sufficient for RBA values, then the limit dose would have to be above 400 μ M (e.g., 1 mM). Decreasing

the limit dose to 100 μM would limit the sensitivity of the assay to RBA values that cover approximately four orders of magnitude.

For the purpose of screening, it is proposed that the limit dose be 1 mM and that a concentration range from 1 mM to 1 nM, in 10-fold increments, be used. However, if it is suspected that the test substance may bind more strongly to the ER than 17 β -estradiol, the dose range should extend from 10 pM to 10 μM in 10-fold increments.

For relatively insoluble substances, the highest dose should be at the limit of solubility and the concentrations tested should be in 10-fold increments. Testing at concentrations that precipitate in the test medium should be avoided to minimize false positive results associated with the non-specific interaction of the precipitate with the ER (Gray et al., 1997).

12.2.5 Solvent and Positive Controls

Concurrent negative, solvent, and positive controls must be included in each experiment. The negative control contains all the reagents of the test system, except the assay solvent, which is replaced with a known non-reactive material, such as water. This sample is processed with treated samples and other control samples to ensure the solvent does not interact with the test system. The solvent control consists of all the reagents of the test system, including the solvent, and should be tested at the highest concentration that is added with the test substance. A positive control substance is included to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. The volume of materials in the ER assay control tubes should equal that of ER assay tubes containing test substance and reference estrogen. Since the RBA for the reference estrogen, 17 β -estradiol, is set at 100, it is recommended that a substance (e.g., tamoxifen, coumestrol) that induces an RBA value between two and three orders of magnitude lower be used as the positive control. The median RBA values of tamoxifen and coumestrol in the RUC assay are reported to be 3.1 and 1.9, respectively (**Appendix D**). If metabolic activation is included in the experimental protocol, then a positive control requiring metabolic activation will need to be included in each experiment to demonstrate the adequacy of the exposure conditions. An appropriate positive control for such studies has not yet been identified.

12.2.6 Within Test Replicates

The IC₅₀ value of the reference compound (i.e., 17 β -estradiol), the positive control, and each test substance should be based on triplicate measurements at each dose level.

12.2.7 Dose Spacing

Generally, to obtain a binding curve, the concentrations of the reference estrogen and the test substances should be spaced by one order of magnitude (i.e., 1 nM, 10 nM, etc.) over the concentration range of interest (1 nM to 1 mM). This results in testing seven concentrations of the test substance in each test. If the range of doses is reduced, then equivalent spacing (e.g., half-log doses) of the seven doses over the smaller dose range should be used.

12.2.8 Data Analysis

Following the measurement of saturation binding of radiolabeled 17 β -estradiol to the ER, and after correcting for nonspecific binding, the binding of 17 β -estradiol is plotted against the log of the concentration of radiolabeled 17 β -estradiol. The curve is analyzed with nonlinear regression techniques to determine B_{max} and K_d. Although a Scatchard analysis (Scatchard, 1949) is frequently used to obtain the K_d, this method has many disadvantages and is not recommended as the primary method (see **Section 2**). Competitive binding experiments use a constant concentration of radiolabeled 17 β -estradiol to measure its displacement from the ER by varying concentrations of reference estrogen or test substance. These data are analyzed by nonlinear regression analysis to determine the IC₅₀ of the test substance or the reference estrogen. The RBA value for the test substance is calculated by dividing the IC₅₀ for 17 β -estradiol (or other reference estrogen) by the IC₅₀ of the test substance and multiplying the result by 100. The K_i is calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973) as a means of assessing the reproducibility of the data from experiment to experiment.

$$K_i = \frac{IC_{50}}{1 + \frac{[Radiolabeled\ 17\beta\text{-estradiol}]}{K_d}}$$

12.2.9 Assay Acceptance Criteria

An assay will be considered acceptable for evaluation if the following conditions are met:

- The unlabeled 17 β -estradiol standard curve demonstrates that increasing concentrations of unlabeled 17 β -estradiol can displace ^3H -17 β -estradiol, and that the IC_{50} value for 17 β -estradiol is approximately equal to the molar concentration of ^3H -17 β -estradiol plus the K_d (determined by non-linear regression and viewed by a Scatchard plot);
- The K_d and IC_{50} values for the unlabeled 17 β -estradiol standard curve are within the confidence limits for historical data;
- The ratio of total binding in the absence of competitor to the amount of ^3H -17 β -estradiol added per assay tube is not greater than 10%;
- The K_i , IC_{50} , and RBA values for the concurrent positive control are within the confidence limits for historical data; and
- The solvent control, at the concentration used, did not alter the sensitivity or reliability of the assay.

12.2.10 Evaluation and Interpretation of Results

A substance is classified as positive for binding to the ER if an IC_{50} value can be obtained and an RBA can be calculated. If an IC_{50} cannot be obtained after testing to the limit dose or the highest dose possible, the test substance is usually classified as being “negative” for *in vitro* ER binding. However, due to solubility constraints (for example), some test substances might induce a significant reduction in binding without achieving at least a 50% reduction in the binding of the reference estrogen to the ER. Until additional information becomes available about the significance of this category of dose response curves, such responses should be noted and the substances classified appropriately (e.g., “equivocal”) for the test.

12.2.11 Test Report

At a minimum, the test report must include the following information:

Test substance:

- Name, chemical structure, and CASRN, if known;
- Physical nature (solid or liquid), and purity, if known; and

- Physicochemical properties relevant to the study (e.g., solubility, stability, volatility).

Solvent:

- Justification for choice of solvent if other than water or ethanol; and
- Information to demonstrate that the solvent, if other than an established solvent, does not bind to, or otherwise affect, the ER.

Estrogen receptor:

- Type and source of ER (if from a commercial source, the supplier must be identified);
- Isolation procedure or method for making construct if isolated protein used;
- Protein concentration of ER preparation; and
- Method for storage of ER, if applicable.

Test conditions:

- K_d of the reference estrogen;
- Rationale for the concentration of the reference estrogen;
- Composition of buffer(s) used;
- Concentration range of test substance, with justification;
- Volume of vehicle used to dissolve the test substance and the volume of test substance added;
- Incubation time and temperature;
- Type and composition of metabolic activation system, if added;
- Concentration range of positive and solvent/vehicle controls;
- Method used to separate free reference estrogen, if applicable;
- Method for analyzing bound reference substance;
- Methods used to determine K_i and IC_{50} values; and
- Statistical methods used, if any.

Results:

- Extent of precipitation of test substance;
- The solvent control response compared to the negative control;

- IC data for each replicate at each dose level for all substances, including confidence levels or other measure of intra-dose repeatability;
- Calculated K_i and IC_{50} values and confidence limits for 17 β -estradiol, the positive control, and the test substance; and
- Calculated RBA values for the positive control and the test substance.

Discussion of the results:

- Historical K_i and IC_{50} values for the reference estrogen, including ranges, means, and standard deviations;
- Reproducibility of the K_i and IC_{50} values of the reference estrogen, compared to historical data;
- Historical solvent and positive control data with ranges, means, and standard deviations;
- Reproducibility of the K_i and IC_{50} /RBA values for the positive control substance, compared to historical data; and
- The nature of the binding dose response relationship for the test substance.

Conclusion:

- Classification of test substance with regard to *in vitro* ER binding activity.

12.2.12 Replicate Studies

Generally, replicate studies are not mandated for screening assays. However, in situations where questionable data are obtained (i.e., the IC_{50} value is not well defined, “equivocal” results are obtained), additional testing using a more narrow range of test substance concentrations to clarify the results of the primary test would be prudent.

12.3 Standardization of ER Binding Assays for Validation

Appendix B provides *in vitro* ER binding assay protocols (or standard operating procedures) provided by five investigators and one protocol for use with a commercially available ER binding test kit (Pan Vera Corporation, Madison, WI, USA). The assay protocols (as titled by the investigator) included in **Appendix B** are:

- The Estrogen Receptor Competitive Binding Assay Using Rat Uterine Cytosol, as provided by Dr. Susan Laws, U.S. EPA, NHEERL, Research Triangle Park, NC, and Mr. Gary Timm, U.S. EPA, Washington, DC.
- The Competitive ER Binding MCF-7 Whole Cell Assay, as provided by Dr. Guy Leclercq, Clinique et Laboratoire de Cancerologie Mammaire, Centre des Tumeurs de l'Université Libre de Bruxelles, Brussels, Belgium.
- The Fluorescence Polarization Assay of the Competitive Binding of Ligands to Estrogen-Receptor Complexes, as provided by Dr William Allworth, Department of Chemistry, University of New Orleans, New Orleans, LA.
- The Competitive Ligand Binding Assay, as provided by Dr. Timothy Zacharewski, Dept. of Biochemistry, Michigan State University, Lansing, MI.
- The Rat Estrogen Receptor Equilibrium Exchange Assay, as provided by Dr. Weida Tong, Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR.

Inspection of these protocols provides a perspective on how various assays are conducted by different investigators.

12.3.1 Example Recommended General Protocol for Measuring ER Binding Using the RUC Assay

An example *in vitro* ER binding assay test method protocol is provided in the **Annex** to **Section 12** (designated Annex protocol). This recommended general (as opposed to laboratory-specific) protocol for the RUC assay is based on the RUC assay protocol supplied by the U.S. EPA (**Appendix B-5**) and on information obtained from expert U.S. EPA scientists (Drs. S. Laws, R. Cooper, E. Gray) and professional (Drs. J. Pounds, J. Morris) and technical staff at Battelle Pacific Northwest Laboratories. This general protocol takes into account the minimum procedural standards described in **Section 12.2**. Specific differences between the original U.S. EPA protocol and the version provided in the Annex are described in the following sections. The protocol is included solely to provide guidance to investigators interested in developing comparable laboratory-specific protocols; it has not been used to generate experimental data.

12.3.2 Preparation of Rat Uterine Cytosol (Annex Section 3, Appendix B-4 Section I)

- Animal Use: Consistent with U.S. Government policy, a statement has been added to the Annex protocol that all studies utilizing animals should be approved by the Institutional Animal Care and Use Committee (IACUC) or its equivalent.

12.3.3 Standardization of *In Vitro* ER Binding Assays (Annex Section 4, Appendix B-4 Section II)

- Data Analysis: Since the IC_{50} value is a property of the experiment and the K_i a property of the receptor and the test substance, the Annex protocol recommends that the K_i value be calculated and provided, in addition to the RBA value. Calculation and analysis of the K_i value is not considered in the U.S. EPA RUC protocol.
- Standardization Acceptance Criteria: The Annex protocol includes an assessment against published and historical data of the K_i , as well as the more typical IC_{50} values, for unlabeled 17 β -estradiol. The K_i value is not considered in the U.S. EPA RUC protocol.
- Standardization Substances: The U.S. EPA RUC protocol proposes that DES, estrone, and ethynyl estradiol be used as positive and R1881 as negative ER binding substances during efforts to standardize the performance of the RUC assay in the laboratory. After consideration of the data provided in **Appendix D** (see **Table 12-1**), the Annex protocol proposes that tamoxifen and coumestrol be used as positive and β -sitosterol as negative ER binding substances for this purpose. Selection of these two positive ER binding substances is based on a desire to use substances that induce an RBA value between two and three orders of magnitude lower than the reference estrogen to assure the sensitivity of the assay. β -Sitosterol was selected as the negative ER binding substance because of the extent of available data (**Table 12-1**). The Annex protocol includes an assessment, where feasible, against published and historical data of the K_i , as well as IC_{50} and RBA values, for these substances.

12.3.4 *In Vitro* ER Competitive Binding Assay Methodology (Annex Section 5, Appendix B-4 Section III)

- Replicate Assay Tubes: The Annex RUC protocol specifies the use of triplicate (rather than duplicate) assay tubes per concentration tested. The additional assay tube will increase the

accuracy of each measured response, and thus the accuracy of the calculated IC₅₀ and RBA values.

- **Solvent and Positive Controls:** The Annex RUC protocol states that when testing substances for their ability to bind to the ER, concurrent negative, solvent and positive controls should be included in each experiment. The U.S EPA RUC protocol includes a solvent control and a substance without ER binding activity as a negative control substance (tested at a single maximal concentration), but does not include negative or positive controls. A positive control substance is included in the Annex RUC protocol to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. While the reference estrogen provides some aspects of a positive control (i.e., it demonstrates the functionality of the assay), it does not allow for an evaluation of the variability in RBA values across experiments. The Annex RUC protocol does not recommend the routine use of a negative control substance.
- **Stock Solutions:** The Annex RUC protocol specifies that test substances be dissolved in water or in a solvent that is miscible with water. For substances not sufficiently water soluble, absolute ethanol or DMSO are proposed as solvents. Other solvents may be used as long as it can be demonstrated that they do not interact with the test system. The U.S. EPA RUC protocol specifies the use of absolute ethanol only. Choice of solvent should depend on which solvent allows the maximum testable concentration of the test substance.
- **Serial Dilutions:** In the Annex RUC protocol, it is proposed for the purpose of screening for ED substances that the limit dose be 1 mM. This limit dose (unless precluded by solubility constraints) allows for the detection of an IC₅₀ value up to five orders of magnitude below that for 17 β -estradiol, the reference estrogen. The U.S EPA RUC protocol specifies an upper limit dose of 0.3 mM.
- **Evaluation and Interpretation of Results:** In the Annex protocol, criteria for specifying a test substance as positive, negative, or equivocal for binding to the ER are provided. The U.S. EPA RUC protocol provides more limited guidance and does not consider the possibility of “equivocal” responses.
- **Test Report:** The Annex protocol specifies the information to be included in the Test Report; the U.S. EPA RUC protocol does not. Such guidance ensures that the test reports contain all pertinent information.

- Replicate Studies: The Annex protocol specifies situations for conducting replicate studies (i.e., in situations where questionable data are obtained) to clarify the results of the primary test. The U.S. EPA RUC protocol does not address the issue of replicate studies.

12.4 Recommended List of Substances to be Used for Validation of *In Vitro* ER Binding Assays

Table 12-1 provides a recommended list of substances to be used in the assessment of the reliability and comparative performance of existing or new *in vitro* ER binding assays. A number of factors were considered in developing this list, including the number of times the substance had been tested in the RUC assay, the median RBA value of the substance in the RUC assay, and the extent of concordance of the RUC median RBA value with values obtained for the same substance in other *in vitro* ER binding assays. Because the number of substances tested by multiple laboratories in the RUC assay was insufficient to generate the desired number of substances for consideration, selection of additional substances was based on the availability and concordance of multiple test data among the 13 other *in vitro* ER binding assays considered in this BRD, and the resulting median RBA value across assays. The selected substances were sorted according to their median RBA values. Because the spread of values extended over seven orders of magnitude, ranging from 400 to 0.0001, the substances were sorted into six categories in log decrements: ≥ 10 , <10 -1; <1 -0.1; <0.01 -0.1, <0.01 -0.001; <0.001 . Weakly-binding substances (RBA values <0.001) were difficult to identify because they were not always consistently positive in tests within an assay or between different assays. Also included were substances classified as "negative" for ER binding based on the lack of a positive response in multiple assays when tested at dose levels of at least 1 mM in at least one assay.

Table 12-1 Recommended Substances for Validation of *In Vitro* ER Binding Assays

Classification RBA Range	Substance	CASRN	Median RBA Value	Chemical Class	No. Assays in which Tested^a	No. Assays with a Positive^a
	DES	56-53-1	200*	Stilbene	14	14
	4-Hydroxytamoxifen	68047-06-3	175*	Triphenylethylene	13	13
	Estrone	53-16-7	48*	Steroid, phenolic	13	13
	Zearalenone	17924-92-4	44*	Acid lactone	10	10
	Estriol	50-27-1	14*	Steroid, phenolic	12	12
<10 to 1	2',4',6',-Trichloro-4-biphenylol	14962-28-8	3.6**	PCB	4	4
	Tamoxifen	10540-29-1	3.1*	Stilbene	14	14
	Bisphenol C2	14868-03-2	2.6*	Diphenylalkane	3	3
	Coumestrol	479-13-0	1.9*	Benzopyrone	11	11
	Mestranol	72-33-3	1.3*	Steroid, nonphenolic	2	2
<1 to 0.1	Nafoxidine	1845-11-0	0.72**	Triphenylethylene	6	5
	Genistein	446-72-0	0.56*	Flavone	11	11
	Norethynodrel	68-23-5	0.22*	Steroid, nonphenolic	3	3
	4- <i>tert</i> -Octylphenol	140-66-9	0.20*	Phenol	9	9
	Phloretin	60-82-2	0.069*	Flavone	3	3
<0.1 to 0.01	Bisphenol A	80-05-7	0.056*	Diphenylalkane	12	12
	Kepone	143-50-0	0.027*	Organochlorine	10	9
	Kaempferol	520-18-3	0.025*	Flavone	3	3
	5 -Dihydrotestosterone	521-18-6	0.014*	Steroid, nonphenolic	9	9

Classification RBA Range	Substance	CASRN	Median RBA Value	Chemical Class	No. Assays in which Tested ^a	No. Assays with a Positive ^a
	<i>o,p'</i> -DDT	789-02-6	0.013*	Organochlorine	12	10
<0.01 to 0.001	Naringenin	480-41-1	0.008*	Flavone	8	6
	4-Androstenedione	63-05-8	0.007**	Steroid, nonphenolic	3	1
	4-Chloro-4'-biphenylol	28034-99-3	0.007*	PCB	2	2
	4-Octylphenol	1806-26-4	0.005*	Phenol	5	4
	Methoxychlor	72-43-5	0.001*	Organochlorine	9	5
<0.001 to 0.0001	4- <i>tert</i> -Butylphenol	98-54-4	0.0009*	Phenol	1	1
	Morin	480-16-0	0.0005*	Flavone	1	1
	<i>p,p'</i> -DDT	50-29-3	0.0003*	Organochlorine	6	2
	Progesterone	57-83-0	0.0003*	Steroid, nonphenolic	2	1
	Atrazine	1912-24-9	0.0003*	Aromatic amine	6	1
Negative	Simazine	122-34-9	HTD-2000 μ M	Triazine	6	0
	-Sitosterol	83-46-5	HTD-1000 μ M	Steroid, nonphenolic	8	0
	Diethylhexyl phthalate	117-81-7	HTD-5000 μ M	Phthalate	1	0

Abbreviations: RUC = Rat uterine cytosol, DES = diethylstilbestrol; PCB = polychlorinated biphenyl; DDT = dichlorodiphenyltrichloroethane; HTD= Highest tested dose

^aNegative test results at maximum tested concentrations <100 μ M were excluded from consideration.

*Median RBA value for positive RUC tests

**Not tested in RUC, median RBA value across all other assays (positive tests only)

Five substances were selected for each RBA category and three for the negative category group. To ensure that each RBA category contained a representative sampling of chemical classes, selection was based on the chemical class to which the substance belongs and whether it was representative of a chemical class used in commerce or found in the environment, and whether it is commercially available. The latter criterion was based on whether the substance could be located in a chemical supply catalogue.

The chemical classes of the substances and the number of substances in each class in **Table 12-1** include nonphenolic steroids (6), organochlorines (4), polychlorinated biphenyls (PCBs), including hydroxylated derivatives (2), flavones (5), phenolic steroids (2), phenols (3), diphenylalkanes (2), stilbenes (2), triphenylethylenes (2), an aromatic amine (1), an acid lactone (1), a benzopyrone (1), a phthalate (1), and a triazine (1).

In March 2001, the U.S. EPA provided a list of 25 substances proposed for testing by Battelle Pacific Northwest (Richland, Washington) in an *in vitro* ER binding RUC assay procedure. In January 2002, EPA provided a modified list of 22 substances. Data generated by the U.S. EPA-sponsored study will be used to validate two QSAR models presently being developed by scientists at the FDA NCTR and by Dr. Mekenyan in Bulgaria. The 22 substances were chosen based on the availability of historical data demonstrating the *in vitro* ER binding affinity, ease of purchase at a purity of >98%, and the lack of extensive health and safety requirements for use (S. Laws, personal communication). Representation of all chemical classes was not a high priority.

The range of binding affinity for the chemicals included those expected to be high affinity binders (nM) to low affinity binders (μ M and mM) to non-binders. The substances on the U.S. EPA list (**Table 12-2**) were compared to those recommended here. The U.S. EPA list lacks substances in certain chemical classes, such as PCBs and organochlorines, which have been demonstrated to bind to the ER (**Appendix D**). Since these two chemical classes are ubiquitous in the environment, representative substances were included in the list of substances recommended for validation in this BRD. However, due to possible concern

Table 12-2 List of Substances Being Tested in the *In Vitro* ER Binding RUC Assay by Battelle

Classification RBA Range	Substances	RUC Median RBA Value	No. Times Tested in RUC Assay	Included in Recommended List in BRD
10	Meso Hexestrol	300	2	No
	17 -Ethinyl estradiol	173	4	No
	17 -Estradiol	Set at 100	Reference estrogen	Yes
	Estrone	48	4	No
	17 -Estradiol	26.5	2	No
	Coumestrol	1.9	2	Yes
	Tamoxifen citrate	1.62	1	No
1 to 0.1	Clomiphene citrate	0.72	1	No
	Norethynodrel	0.22	2	Yes
	Bisphenol B	0.12	2	No
<0.1 to 0.01	Bisphenol A	0.056	5	Yes
	4-Nonylphenol	0.033	10	No
	Kaempferol	0.025	1	Yes
	Daidzein	0.023	1	No
<0.01 to 0.001	4-Cumylphenol	0.005	1	No
<0.001 to 0.0001	Ethyl 4-hydroxybenzoate	0.0006	1	No
	Morin	0.0005	1	Yes
	Progesterone	0.0003	1/3*	Yes
	2- <i>sec</i> -butylphenol	0.0003	1	No
	Phenolphthalin	0.0002	1	No
Negative	Corticosterone	Negative (100 μ M)	1	No
	2,4,5-Trichlorophenoxy-acetic acid	Negative (1000 μ M)	1	No

*The substance was positive in one of three tests.

about the disposal of the PCB congeners (concentrations in excess of 50 ppm require special disposal procedures), inclusion of this chemical class should be considered further.

Eight substances presently being tested by Battelle were not included in the validation list because of limited published data on their activity in the RUC assay (**Appendix D**). These are tamoxifen citrate, clomiphene citrate, 4-cumylphenol, ethyl-4-hydroxybenzoate, 2-*sec*-

butylphenol, phenolphthalin, trichloroacetic acid, bisphenol B, corticosterone, and 2,4,5-trichlorophenoxy-acetic acid. Rather than tamoxifen citrate, tamoxifen is recommended (the RBA values are similar) because it has been tested 21 times. 4-Nonylphenol was not selected as much of the published reports used an undefined nonylphenol or a mixture of nonylphenol isomers. Among the substances with the highest binding affinity (RBA values ≥ 10), both 17 β -ethinyl estradiol and meso-hexestrol were considered for inclusion in the proposed list of substances to be used in validation studies, but since DES and 4-hydroxytamoxifen had been tested in a wider range of assays, they were selected. Genistein was selected over daidzein in the 1 to 0.1 RBA value range because it had been tested more frequently. Although morin had only been tested once, it was included in the recommended list since it was considered desirable to have one representative flavone, where possible, in each RBA value range. Corticosterone and 2,4,5-trichlorophenoxy-acetic acid, the two substances categorized as negative for ER binding in the Battelle list, were excluded from the recommended list due to limited data (i.e., the highest dose tested for corticosterone in any study was 100 μ M; there was only a single study on 2,4,5-trichlorophenoxy-acetic acid).

In a validation study, it is important to include substances that cover the range of possible responses without necessarily having the same numbers of substances in each of the artificially defined categories. However, for balance, it would seem that it would be desirable to have equal numbers of substances in each RBA category. When available, the results from the Battelle study might be used to modify the recommended list.

12.5 Conclusions and Recommendations

Currently, there are no published guidelines for conducting *in vitro* ER binding studies, and no formal validation studies to assess the reliability or performance of ER binding assays have been performed. To support the further development and characterization of *in vitro* ER binding assays, minimum procedural standards for such assays and a recommended list of test substances for use in validation studies are provided. The minimum procedural standards and recommended test substances are based on a comparative evaluation of the 14 *in vitro* ER binding assays summarized and evaluated in this BRD. The RUC assay, which has been the most widely used

method for identifying substances with ER binding activity, is proposed as the standard against which new tests should be evaluated.

The minimum procedural standards consider methods for determining the K_d of the reference estrogen, methods for test substance preparation, the concentration range of the test substance to evaluate (including the limit dose), the use of solvent and positive controls, the number of replicates to use per test substance concentration, dose spacing, data analysis, assay acceptance criteria, evaluation and interpretation of results, minimal information to include in the test report, and the potential need for replicate studies. These minimum procedural standards are provided to ensure that *in vitro* ER binding studies will be conducted to the same minimal standards.

A suggested general protocol for measuring ER binding using the RUC assay was developed based on a submitted U.S. EPA protocol. Aspects of the RUC assay protocol presented included preparation of rat uterine cytosol, standardization of the assay, the saturation radioligand binding assay, the ER competitive binding assay, considerations for standardizing ER binding assays, ER competitive binding assay methodology, preparation of TEDG assay buffer, preparation of the radiolabeled reference estrogen, preparation of unlabeled reference estrogen, selection of ER concentration and assay volume, preparation of the reference estrogen for the standard curve and nonspecific binding measurements, preparation of test substances, preparation of ER assay tubes, preparation of the HAP slurry, separation of ER-bound radiolabeled 17 β -estradiol from free labeled and unlabeled 17 β -estradiol, extraction and quantification of the radiolabeled reference estrogen bound to ER, data analysis, and report specifications. It is hoped that such guidance will help investigators in their development of laboratory specific protocols for conducting validation studies on *in vitro* ER binding assays.

A number of factors were considered in developing a list of substances to be used in validation efforts, including the number of times the substance had been tested in the RUC assay, the median RBA value of the substance in the RUC assay, and the extent of concordance of the RUC median RBA value with values obtained for the same substance in other *in vitro* ER binding assays. Because the number of substances tested by multiple laboratories in the RUC assay was insufficient to generate the desired number of substances for consideration, selection of

additional substances was based on the availability and concordance of multiple test data among the 13 other *in vitro* ER binding assays considered in this BRD. The selected substances were sorted according to their median RBA values, over seven orders of magnitude, ranging from 400 to 0.0001. Weakly-binding substances (RBA values <0.001) were difficult to identify because they were not always consistently positive in tests within an assay or using different assays. Also included were substances classified as "negative" for ER binding based on the lack of a positive response in multiple assays when tested at doses of at least 1 mM. Five substances were selected for each RBA category and three for the negative category group. To ensure that each RBA category contained a representative sampling of chemical classes, selection was based on the chemical class to which the substance belongs, whether it was representative of a chemical class used in commerce or found in the environment, and whether the substance is commercially available. The latter criterion was based on whether the substance could be located in a chemical supply catalogue.

The resulting list of 33 substances was compared with the U.S. EPA list of 22 substances to be tested in an RUC assay procedure by Battelle. The U.S. EPA list lacks substances in certain chemical classes, such as PCBs and organochlorines, which have been demonstrated to bind to the ER. Since these two chemical classes are ubiquitous in the environment, representative substances were included in the proposed list of validation substances. Eight of the substances on the U.S. EPA list were not considered because of limited published data on their activity in the RUC assay.

Example Protocol for the *In Vitro* Estrogen Receptor (ER) Competitive Binding Assay Using Rat Uterine Cytosol (RUC)

1.0 Purpose of Assay: This assay is used to determine the relative binding affinities of test substances for the estrogen receptor, which is comprised of the ER α and ER β subtypes, compared to 17 β -estradiol. The primary purpose for this assay is as a screening tool to detect substances with possible estrogenic or anti-estrogenic properties. This example protocol is intended to serve as a guide for producing laboratory specific protocols using this and related assays.

2.0 Terminology:

DES: Diethylstilbestrol

DMSO: Dimethyl sulfoxide

^3H -17 β -estradiol: 17 β -estradiol radiolabeled with tritiated thymidine

HAP: Hydroxylapatite

TEDG buffer: 10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.4

3.0 Preparation of Rat Uterine Cytosol

Note: All studies utilizing animals should be approved prior to implementation by the Institutional Animal Care and Use Committee (IACUC) or its equivalent.

3.1 TEDG Buffer

Prepare buffer; dithiothreitol is added immediately prior to use.

3.2 Collect Uteri

Collect uteri from female rats ovariectomized seven to ten days prior to being humanely killed. Quickly trim fat and mesentery from the uterus. Weigh and record

the weight of each uterus. Uteri may be used immediately or rapidly frozen in liquid nitrogen, and stored at -80°C for up to three months.

Note: Consistency for all assays should be maintained with respect to the age and strain of the animals used.

3.3 Uterine Cytosol

3.2.1 Weigh trimmed uterus and place in ice-cold TEDG buffer at a ratio of 0.1 g of tissue per 1.0 mL TEDG buffer. Homogenize the tissue using an appropriate homogenizer (5-second bursts).

Note: Cool the homogenizer probe prior to homogenizing each sample by placing the probe in ice-cold TEDG buffer. The homogenization tube should be kept in an ice-cold water bath during the homogenizing process.

3.2.2 Transfer the homogenate to pre-cooled centrifuge tubes and centrifuge for 10 minutes at $2,500 \times g$ at 4°C . The pellet contains the nuclear fraction and the supernatant the ER containing cytosol.

3.2.3 Transfer the supernatant to pre-cooled ultracentrifuge tubes, and centrifuge at $105,000 \times g$ for 60 minutes at 4°C .

3.2.4 Combine the cytosol containing ER supernatants from uteri collected the same day and aliquot for immediate use in ER binding assay or for storage at -80°C .

Note: The cytosol can be stored frozen at -80°C for 1 month prior to use in ER binding assay. Do not thaw and re-freeze the cytosol.

3.2.5 Determine the protein content for each batch of cytosol using an appropriate method.

Note: The dithiothreitol in the buffer is not compatible with the Pierce BCA Protein Assay. Typical protein values are 4 -7 mg/mL.

4.0 Standardization of ER Competitive Binding Assays

Prior to routinely conducting the ER competitive binding assays, the methods should be standardized within each laboratory. This can be accomplished in two steps. First, a series of saturation radioligand binding assays should be conducted to demonstrate ER specificity and saturation. Nonlinear regression analysis of these data (e.g., McPherson, 1985; c1997; Motulsky, 1995) and subsequent Scatchard plots will document ER binding affinity (K_d) and the number of receptors (B_{max}). Second, a series of ER competitive binding assays should be conducted using substances (e.g., 17 β -estradiol, DES, estrone) with known affinities for the ER. Comparison of IC_{50} values (e.g., the concentration of a substance that inhibits 3H -17 β -estradiol binding by 50%) from these assays with reported values in the literature will assist in documenting that the methods are appropriate for routine use in the laboratory.

4.1 Saturation Radioligand Binding Assay: ER saturation binding experiments measure total, non-specific, and specific binding of increasing concentrations of 3H -17 β -estradiol under conditions of equilibrium. A graph of specific 3H -17 β -estradiol binding versus radioligand concentration should reach a plateau for maximum specific binding indicative of saturation of the ER with the radioligand. In addition, analysis of the data should document the binding of the 3H -17 β -estradiol to a single, high-affinity binding site (e.g., $K_d = 0.05$ to 0.1 nM and a linear Scatchard plot).

Although several saturation radioligand assays may need to be conducted before an optimal saturation curve, K_d , and B_{max} are achieved, a good starting point is to use enough cytosol to provide 50 to 100 μ g protein per assay tube. The concentration for 3H -17 β -estradiol should range from 0.03 to 3.0 nM in a total assay volume of 0.5 mL. Non-specific binding should be determined by adding unlabeled 17 β -estradiol at 100x the concentration of radiolabeled 17 β -estradiol. Analysis of these data should use a non-linear regression analysis (e.g., McPherson, 1985; c1997; Motulsky,

1995) with a final display of the data as a Scatchard plot. Rat uterine cytosol prepared using this protocol will typically yield a K_d of 0.05 to 0.1 nM and B_{max} of 36 -44 fmol ER/100 μ g protein (equivalent to 0.072 to 0.088 nM ER, respectively, when 100 μ g protein used in total assay volume of 0.5 mL).

An example of a saturation assay worksheet using increasing concentrations of radioligand is provided in **Table 1**.

Note: For this example, a stock solution of unlabeled 17 β -estradiol is prepared in absolute ethanol, with all serial dilutions prepared in assay buffer. All concentrations of 3 H-17 β -estradiol are prepared in assay buffer.

Table 1 Typical 17 β -Estradiol Saturation Assay

Tube Number	3 H-17 β -Estradiol			Unlabeled 17 β -Estradiol			Buffer	Cytosol
	Initial Conc. (nM)	Vol. (μ L)	Final Conc. (nM)	Initial Conc. (nM)	Vol. (μ L)	Final Conc. (nM)	Vol. (μ L)	Vol. (μ L)
1	0.3	50	0.03	-			350	100
2	0.3	50	0.03	-			350	100
3	0.3	50	0.03	-			350	100
4	0.6	50	0.06	-			350	100
5	0.6	50	0.06	-			350	100
6	0.6	50	0.06	-			350	100
7	0.8	50	0.08	-			350	100
8	0.8	50	0.08	-			350	100
9	0.8	50	0.08	-			350	100
10	1.0	50	0.1	-			350	100
11	1.0	50	0.1	-			350	100
12	1.0	50	0.1	-			350	100
13	3.0	50	0.3	-			350	100
14	3.0	50	0.3	-			350	100
15	3.0	50	0.3	-			350	100
16	6.0	50	0.6	-			350	100
17	6.0	50	0.6	-			350	100
18	6.0	50	0.6	-			350	100
19	10	50	1	-			350	100
20	10	50	1	-			350	100
21	10	50	1	-			350	100

Tube Number	³ H-17 β -Estradiol			Unlabeled 17 β -Estradiol			Buffer	Cytosol
	Initial Conc. (nM)	Vol. (μ L)	Final Conc. (nM)	Initial Conc. (nM)	Vol. (μ L)	Final Conc. (nM)	Vol. (μ L)	Vol. (μ L)
22	30	50	3	-			350	100
23	30	50	3	-			350	100
24	30	50	3	-			350	100
25	0.3	50	0.03	30	50	3	300	100
26	0.3	50	0.03	30	50	3	300	100
27	0.3	50	0.03	30	50	3	300	100
28	0.6	50	0.06	60	50	6	300	100
29	0.6	50	0.06	60	50	6	300	100
30	0.6	50	0.06	60	50	6	300	100
31	0.8	50	0.08	80	50	8	300	100
32	0.8	50	0.08	80	50	8	300	100
33	0.8	50	0.08	80	50	8	300	100
34	1.0	50	0.1	100	50	10	300	100
35	1.0	50	0.1	100	50	10	300	100
36	1.0	50	0.1	100	50	10	300	100
37	3.0	50	0.3	300	50	30	300	100
38	3.0	50	0.3	300	50	30	300	100
39	3.0	50	0.3	300	50	30	300	100
40	6.0	50	0.6	600	50	60	300	100
41	6.0	50	0.6	600	50	60	300	100
42	6.0	50	0.6	600	50	60	300	100
43	10	50	1	1000	50	100	300	100
44	10	50	1	1000	50	100	300	100
45	10	50	1	1000	50	100	300	100
46	30	50	3	3000	50	300	300	100
47	30	50	3	3000	50	300	300	100
48	30	50	3	3000	50	300	300	100
49	0.3	50	0.03	³ H-17 -estradiol only, for determining total dpms				
50	0.3	50	0.03	³ H-17 -estradiol only, for determining total dpms				
51	0.3	50	0.03	³ H-17 -estradiol only, for determining total dpms				
52	0.6	50	0.06	³ H-17 -estradiol only, for determining total dpms				
53	0.6	50	0.06	³ H-17 -estradiol only, for determining total dpms				
54	0.6	50	0.06	³ H-17 -estradiol only, for determining total dpms				
55	0.8	50	0.08	³ H-17 -estradiol only, for determining total dpms				

Tube Number	³ H-17 β -Estradiol			Unlabeled 17 β -Estradiol			Buffer	Cytosol
	Initial Conc. (nM)	Vol. (μ L)	Final Conc. (nM)	Initial Conc. (nM)	Vol. (μ L)	Final Conc. (nM)	Vol. (μ L)	Vol. (μ L)
56	0.8	50	0.08	³ H-17 -estradiol only, for determining total				
57	0.8	50	0.08	³ H-17 -estradiol only, for determining total dpms				
58	1.0	50	0.1	³ H-17 -estradiol only, for determining total dpms				
59	1.0	50	0.1	³ H-17 -estradiol only, for determining total dpms				
60	1.0	50	0.1	³ H-17 -estradiol only, for determining total dpms				
61	3.0	50	0.3	³ H-17 -estradiol only, for determining total dpms				
62	3.0	50	0.3	³ H-17 -estradiol only, for determining total dpms				
63	3.0	50	0.3	³ H-17 -estradiol only, for determining total dpms				
64	6.0	50	0.6	³ H-17 -estradiol only, for determining total dpms				
65	6.0	50	0.6	³ H-17 -estradiol only, for determining total dpms				
66	6.0	50	0.6	³ H-17 -estradiol only, for determining total dpms				
67	10	50	1	³ H-17 -estradiol only, for determining total dpms				
68	10	50	1	³ H-17 -estradiol only, for determining total dpms				
69	10	50	1	³ H-17 -estradiol only, for determining total dpms				
70	30	50	3	³ H-17 -estradiol only, for determining total dpms				
71	30	50	3	³ H-17 -estradiol only, for determining total dpms				
72	30	50	3	³ H-17 -estradiol only, for determining total dpms				

Abbreviations: Conc. = concentration; Vol. = volume; dpms = disintegrations per minute

4.2 ER Competitive Binding Assay: An ER competitive binding assay measures the binding of a single concentration of ^3H -17 β -estradiol in the presence of increasing concentrations of a test substance. The competitive binding curve is plotted as total ^3H -17 β -estradiol binding versus the concentration (log units) of the competitor. The concentration of the test substance that inhibits 50% of the maximum ^3H -17 β -estradiol binding is the IC_{50} value. Preliminary experiments should evaluate the effect of the ER concentration of the cytosol, assay volume, and ^3H -17 β -estradiol concentration on the IC_{50} calculation using unlabeled 17 β -estradiol. A good starting point for the ER competitive binding assay is to use enough cytosol to provide 50 to 100 μg protein per assay tube, with 0.5 -1.0 nM ^3H -17 β -estradiol in a total assay volume of 0.5 mL. Once assay conditions have been optimized, additional ER competitive binding assays should be conducted to test substances with known affinities for the ER. Such substances include tamoxifen, ethynyl estradiol, coumestrol, and estrone as positive ER binding substances, and R1881 (methyltrienolone) as the negative ER binding substance. Data for the unlabeled 17 β -estradiol standard curve and each validation substance should be plotted as the percent ^3H -17 β -estradiol bound versus the molar concentration (log) of competitor. Estimates of IC_{50} values should be determined using appropriate nonlinear curve fitting software (e.g., McPherson, 1985; c1997; Motulsky, 1995). Since the IC_{50} value is a property of the experiment and the K_i a property of the receptor and the test substance, the K_i value should be provided, as well as the RBA value. The K_i value is calculated using the Cheng -Prusoff equation (Cheng and Prusoff, 1973).

When conducting this assay as a screening test for substances with an ability to bind to the ER, concurrent negative, solvent, and positive controls are included in each experiment. The negative control provides assurance that the solvent does not interact with the test system. The solvent should be tested at the highest

concentration that is added with the test substance. A positive control substance (e.g., tamoxifen, coumestrol) is included to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. Recommended concentrations of the test substance and positive controls to use are from 1×10^{-9} to 1×10^{-3} M, in log increments.

4.3 Standardized *In Vitro* ER Competitive Binding Assay Acceptance Criteria

4.3.1 Saturation Assays. In general, when evaluating data from ER saturation assays, the following points should be considered:

- As increasing concentrations of ^3H -17 β -estradiol were used, did the specific binding curve reach a plateau (e.g., was maximum specific binding reached indicating saturation of ER with ligand)?
- Did the data produce a linear Scatchard plot (e.g., non-linear plots generally indicate a problem with the assay such as ligand depletion [concave plot] or incorrect assessment of non-specific binding [convex plot])?
- Is the K_d within an acceptable range (e.g., 0.05 to 0.1 nM)?

Note: Literature values for K_d using rat uterine cytosolic preparations have varied from 0.05 to 0.5 nM. The variation in K_d may be a reflection of different laboratories using radiolabeled estradiol with a wide range of specific activity (e.g., ^3H -17 β -estradiol versus ^{125}I -17 β -estradiol). In addition, publications by Salomonsson et al. (1994) and Kuiper et al. (1997, 1998) suggest that a lower K_d may be observed when assay conditions minimize ligand depletion, and that slightly different K_d values exist for ER α and ER β .

- Are the standard errors for the K_d or B_{max} excessive? If the ratio of either the standard error (SE) of the K_d to the K_d , or the SE of the B_{max} to the B_{max} is much larger than 20%, then the methods for the assay should be re-evaluated.
- Is non-specific binding excessive? The value for non-specific binding should be less than 50% of the total binding.

4.3.2 Competitive Binding Assays. In general, the assay should demonstrate that increasing concentrations of unlabeled 17 β -estradiol can compete with a single concentration of ^3H -17 β -estradiol for binding to the ER. Specific questions to evaluate are as follows:

- As a safeguard against ligand depletion, was the total maximal binding no greater than 10% of the amount of ^3H -17 β -estradiol added per assay tube?
- Were the K_i and IC_{50} values for unlabeled 17 β -estradiol reasonable? The IC_{50} value for unlabeled 17 β -estradiol should be approximately equal to the molar concentration of ^3H -17 β -estradiol used in the assay tube plus the K_d (determined by nonlinear analysis and Scatchard plot of data obtained from saturation radioligand binding assays).
- Were the K_i , IC_{50} , and RBA values for the substance used to validate the performance of the assay reasonable based on published and historical data?
- Was the negative control substance unable to inhibit binding of the radiolabeled 17 β -estradiol?

5.0 ER Competitive Binding Assay: Working Protocol

5.1 Preparation of Assay Buffer

Prepare TEDG buffer without dithiothreitol, adjust to pH 7.4 and store at 4°C. Add dithiothreitol immediately prior to use in assay.

5.2 Preparation of ^3H -17 β -Estradiol

Store at 4 to 5°C in the original container. Obtain the highest specific activity (SA) available from the vendor.

Note: The SA should be adjusted for decay over time.

Dilute the radiolabeled 17 β -estradiol with TEDG buffer. Each assay tube should contain 0.5 to 1 nM final concentration of ^3H -17 β -estradiol.

5.3 Solvent and Positive Controls

When testing substances for their ability to bind to the ER, concurrent negative, solvent, and positive controls should be included in each experiment. The negative control provides assurance that the solvent does not interact with the test system. The solvent should be tested at the highest concentration that is added with the test substance. A positive control substance is included to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. A positive control substance (e.g., tamoxifen, coumestrol) is included to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. The suggested concentration range of the test substance and positive control to test is from 1×10^{-9} to 1×10^{-3} M, in log increments.

5.3 Selection of Receptor Concentration and Assay Volume

5.3.1 Receptor concentration of the cytosol and assay volume per assay tube should be adjusted to minimize the likelihood of ligand depletion (e.g., ligand

depletion occurs when a high percentage of the ^3H -17 β -estradiol is bound to ER causing the concentration of the unbound (*free*) ^3H -17 β -estradiol to significantly differ from the concentration of ^3H -17 β -estradiol that was originally added to the assay tube [Hulme and Birdshall, 1992]). A general rule is to optimize the assay conditions so that the ratio of the total ^3H -17 β -estradiol bound in the absence of competitor, to the total ^3H -17 β -estradiol added to each assay tube, is no more than 10%. Decreasing the amount of cytosolic protein and/or increasing the assay volume will generally lower this ratio. Serial dilutions of the cytosol to obtain 50 to 150 μg protein per assay tube in a total assay volume of 500 μL is a good starting point for determining the optimal ER concentration.

5.4 Preparation of 17 β -Estradiol for the Standard Curve and Non-Specific Binding (NSB)

5.4.1 Standard Curve: A standard curve using unlabeled 17 β -estradiol should be prepared for each ER competitive binding assay. Final concentrations of unlabeled 17 β -estradiol in the assay tubes should range from 1.0×10^{-7} to 1.0×10^{-11} M. Prepare serial dilutions of 17 β -estradiol in absolute ethanol to achieve the final concentrations shown below. Use siliconized glass tubes when preparing the standards. **Table 2** shows recommended concentrations for the unlabeled 17 β -estradiol standard curve.

5.5 Preparation of Test Substances

5.5.1 Stock Solutions: Test substances must be dissolved in water or in a solvent that is miscible with water. For substances not sufficiently water soluble, absolute ethanol or DMSO are proposed as solvents. Preference is given to absolute ethanol compared to DMSO since this solvent has been

used in most of the studies conducted to date. Other solvents may be used as long as it can be demonstrated that they do not interact with the test system.

Table 2 **Example of Preparation Procedure for Unlabeled 17 β -Estradiol Standard Curve**

Concentrations for Unlabeled 17β-Estradiol Standard Curve		
Standards	Initial 17β-Estradiol Concentration (Molar)	*Final 17β-Estradiol Concentration (Molar) in ER Assay Tube
0	0 (ethanol)	0
NSB	5×10^{-6}	1×10^{-7}
S1	5×10^{-7}	1×10^{-8}
S2	5×10^{-8}	1×10^{-9}
S3	1.67×10^{-8}	3.33×10^{-10}
S4	5×10^{-9}	1×10^{-10}
S5	1.67×10^{-9}	3.33×10^{-11}
S6	5×10^{-10}	1×10^{-11}

When 10 μ L of each standard is added to the ER assay tube, the final concentration will be as indicated when the total volume in the ER assay tube is 500 μ L.

Note: Some test substances will not be soluble at this concentration, so adjustments will need to be made in the final concentration of the serial dilution tubes depending upon the solubility characteristics of specific substances.

5.5.2 Prepare serial dilutions of each test substance in the appropriate solvent to yield the final concentrations as indicated below.

Note: For the purpose of screening, it is proposed that the upper limit dose be 1 mM and that a concentration range from 1 mM to 1 nM, in ten-fold increments, be used. If the upper limit dose must be reduced due to solubility constraints, then equivalent spacing (e.g., half-log doses) of the seven doses over the smaller dose range should be used. The serial dilutions shown in **Table 3** are based upon the addition of 10 μ L of each serial dilution of the test substance in a final assay volume of 500 μ L. Other ratios can be used as long as the solvent concentration does not exceed 0.2%.

Table 3 Test Substance Concentrations

Serial Dilutions of Test Substance	Initial Concentration (Molar)	*Final Concentration in ER Assay Tube (Molar)
Concentration 1	5.0×10^{-2}	1.0×10^{-3}
Concentration 2	5.0×10^{-3}	1.0×10^{-4}
Concentration 3	5.0×10^{-4}	1.0×10^{-5}
Concentration 4	5.0×10^{-5}	1.0×10^{-6}
Concentration 5	5.0×10^{-6}	1.0×10^{-7}
Concentration 6	5.0×10^{-7}	1.0×10^{-8}
Concentration 7	5.0×10^{-8}	1.0×10^{-9}

**Final Concentration of test substance in assay tube when 10 μ L of Initial Concentration is used in a total volume of 500 μ L*

5.6 Preparation of ER Assay Tubes

5.6.1 Label 12 x 75 mm round bottom assay tubes (siliconized glass) in triplicate with codes for the untreated negative control, the solvent control, the NSB, seven dose levels of the positive control substance, and seven dose levels of each test substance.

5.6.2 Place assay tubes in ice bath and add the following to each tube:

Components of ER RUC Binding Assay		
50	μ L	Adjust amount of uterine cytosol to provide 50 to 100 μ g protein/assay tube
430	μ L	TEDG Assay Buffer
10	μ L	3 H-17 β -estradiol to yield final concentration of 0.5 to 1.0 nM
10	μ L	Unlabeled 17 β -estradiol, negative control, or test substance
500	μ L	Total volume in each assay tube

5.6.3 Vortex assay tubes.

Note: Make sure that all components are concentrated at the bottom of tube.

If any of the liquid remains on the side of the tube, centrifuge assay tubes for 1 minute at 600 x g (4°C) to concentrate fluid at bottom of tube.

5.6.4 Incubate assay tubes at 4°C for 18 to 20 hours. Assay tubes should be placed on a rotator during the incubation period.

5.7 Preparation of 60% HAP Slurry

5.7.1 Prepare 60% HAP slurry the day before the step to separate the bound and free ^3H -17 β -estradiol, by adding 10 g HAP to 100 mL TEDG buffer and gently mixing. Cap the container and refrigerate (4°C) the HAP slurry overnight (8 to 10 hours). This amount of HAP will generally yield enough slurry for 70 to 100 assay tubes.

5.7.2 Aspirate the supernatant and resuspend the HAP in fresh TEDG buffer. Allow the HAP to settle and repeat the wash step.

5.7.3 After the last wash, resuspend the HAP to a final volume of 60% HAP and 40% buffer. The HAP slurry should be well suspended and ice-cold when used in the separation procedure.

5.8 Separation of Bound ^3H -17 β -Estradiol -ER and Free ^3H -17 β -Estradiol

Note: To minimize dissociation of bound ^3H -17 β -estradiol from the ER during this process, it is extremely important that the buffers and assay tubes be kept ice-cold and that each step be conducted quickly.

5.8.1 Remove ER assay tubes from rotator and place in an ice-water bath. Using an Eppendorf repeating pipet, quickly add 250 μL of HAP slurry (60% in TEDG buffer, well mixed prior to using) to each assay tube.

5.8.2 Vortex the tubes at 5 minute intervals for a total of 15 minutes.

Note: This is best accomplished by vortexing an entire rack of tubes at once.

It is important to keep the assay tubes cold at this point.

5.8.3 Following the incubation period (step 5.8.2), add 2.0 mL of the TEDG buffer, quickly vortex, and centrifuge at 4°C for 10 minutes at 1000 x g.

5.8.4 After centrifugation, immediately decant the supernatant containing the free ³H-17 -estradiol. The HAP pellet will contain the bound ³H-17 -estradiol -estrogen receptors.

Note: This step can be accomplished quickly by placing the assay tubes in a decanting tube racks. All tubes in the rack can be decanted at once, and the tubes immediately placed back in the ice bath.

5.8.5 Add an additional 2.0 mL ice-cold TEDG buffer and vortex briefly to resuspend the pellet. Work quickly and keep assay tubes cold. Centrifuge again at 4°C for 15 minutes at 1000 x g.

5.8.6 Quickly decant and discard the supernatant. Repeat the wash and centrifugation steps once more.

5.8.7 After the final wash, decant the supernatant. Allow the assay tubes to drain briefly for 1 to 5 minutes.

Note: Watch carefully in case the HAP pellet begins to run down the side of assay tube, which may occur if protein concentration in the cytosol is quite low. At this point, the separation of the free ³H-17 -estradiol and bound ³H-17 -estradiol-ER has been completed. Assay tubes may be left at room temperature.

5.9 Extraction and Quantifying ³H-17β-Estradiol bound to ER

5.9.1 Add 1.5 mL of absolute ethanol to each assay tube. Allow the tubes to sit at room temperature for 15 to 20 minutes, vortexing at 5-minute intervals.

5.9.2 Centrifuge the assay tubes for 10 minutes at 1000 x g and 4°C.

5.9.3 Pipet an aliquot (usually 1.0 to 1.5 mL) or decant the supernatant into 20 mL scintillation vials. Add 10 mL scintillation cocktail, cap and shake vial.

Note: If a 1.0 ml aliquot is used for scintillation counting, the DPMs should be adjusted to account for the total radioactivity in 1.5 ml (i.e., DPMs x 1.5 = Total DPMs bound).

5.9.4 Place vials in scintillation counter for determination of DPMs/vial with quench correction.

5.10 Data Analysis

5.10.1 Terminology

- Total ³H-17 β -estradiol: DPMs added to each assay tube (e.g., can be converted to concentration of total ³H-17 β -estradiol used in the ER assay).
- Total (Maximum) Binding: DPMs in the 0 standard tubes.
- Nonspecific Binding: DPMs in the NSB standard (i.e., 100 x excess of unlabeled 17 β -estradiol).
- Specific Binding: DPMs for each concentration of standard or test substance minus the mean DPM of the NSB tubes.

5.10.2 Data Analysis

- i. IC₅₀ calculation: Data for the unlabeled 17 β -estradiol standard curve and each test substance should be plotted as the percentage of ³H-17 β -estradiol bound versus the molar concentration (log) of competitor. Estimates of IC₅₀ values should be determined using appropriate nonlinear curve fitting software.
- ii. Relative Binding Affinity (RBA) values: The RBA values for each test substance and positive control is calculated by dividing the IC₅₀ value for

- 17 -estradiol by the IC_{50} of the test substance or the positive control and expressing the value as a percent (e.g., RBA for 17 -estradiol = 100 %).
- iii. K_i calculation: Calculate the K_i value from the IC_{50} value using the Cheng-Prusoff (1973) equation (Cheng and Prusoff, 1973).

5.11 Assay Acceptance Criteria

- 5.11.1 Unlabeled 17 -estradiol Standard Curve. The assay should demonstrate that increasing concentrations of unlabeled 17 -estradiol can displace 3H -17 -estradiol, and the IC_{50} value for 17 -estradiol should be approximately equal to the molar concentration of 3H -17 -estradiol plus the K_d (determined by non-linear regression and viewed by a Scatchard plot).
- 5.11.2 The K_d and IC_{50} values for the unlabeled 17 -estradiol standard curve should be within the confidence limits for historical data.
- 5.11.3 The ratio of total binding in the absence of competitor to the amount of 3H -17 -estradiol added per assay tube should not be greater than 10%.
- 5.11.4 The K_i , IC_{50} , and RBA values for the concurrent positive control should be within the confidence limits for historical data.
- 5.11.5 The solvent control substance, at the concentration used, should not alter the sensitivity or reliability of the assay.

5.12 Evaluation and Interpretation of Results

A substance is classified as positive for binding to the ER if a K_i and IC_{50} values can be obtained and an RBA value can be calculated. If K_i/IC_{50} values cannot be obtained after testing to the upper limit dose or the highest dose possible, the test substance is classified as “negative” for *in vitro* ER binding. However, due to solubility constraints (for example), some test substances might induce a significant reduction in binding but without achieving at least a 50% reduction in the binding of the

reference estrogen to the ER. Until additional information becomes available about the significance of this category of dose response curves, such responses should be noted and the substances classified appropriately (e.g., “equivocal”) for the test.

5.13 Test Report

The test report must include, but is not limited to, the following information:

5.13.1 Test Substance

- Name, chemical structure, and CASRN, if known;
- Physical nature (solid or liquid), and purity, if known; and
- Physicochemical properties relevant to the study (e.g., solubility, stability, volatility).

5.13.2 Solvent/Vehicle

- Justification for choice of solvent/vehicle if other than water or ethanol;
- Information to demonstrate that the solvent/vehicle, if other than an established solvent, does not bind to, or otherwise affect, the ER.

5.13.3 Estrogen Receptor

- Type and source of ER (if from a commercial source, the supplier must be identified);
- Isolation procedure from tissues, method for making construct, procedure for isolating protein or construction of fusion protein if used;
- Protein concentration of ER preparation; and
- Method for storage of ER, if applicable.

5.13.4 Test Conditions

- K_d of the reference estrogen;
- Rationale for the concentration of the reference estrogen;
- Composition of buffer(s) used;
- Concentration range of test substance, with justification;

- Volume of vehicle used to dissolve test substance and volume of test substance added;
- Incubation time and temperature;
- Type and composition of metabolic activation system, if added;
- Concentration range of positive and solvent/vehicle controls;
- Method used to separate free reference estrogen, if applicable;
- Method for analyzing bound reference substance;
- Methods used to determine K_i and IC_{50} values; and
- Statistical methods used, if any.

5.13.5 Results

- Extent of precipitation of test substance;
- The solvent control response compared to the negative control;
- IC data for each replicate at each dose level for all substances, including confidence levels or other measure of intra-dose repeatability;
- Calculated K_i and IC_{50} values and confidence limits for 17 β -estradiol, the positive control, and the test substance; and
- Calculated RBA values for the positive control and the test substance.

5.13.6 Discussion of the Results

- Historical K_i and IC_{50} values for reference ligand, including ranges, means, and standard deviations;
- Reproducibility of the K_i and IC_{50} values of the reference ligand, compared to historical data;
- Historical positive control data with ranges, means, and standard deviations; and
- Reproducibility of the K_i and IC_{50} values for the positive control substance, compared to historical data.

5.13.7 Conclusion

- Classification of test substance with regard to *in vitro* ER-binding activity.

5.14 Replicate Studies

Generally, replicate studies are not mandated for screening assays. However, in situations where questionable data are obtained (i.e., the IC₅₀ value is not well defined), replicate tests to clarify the results of the primary test would be prudent.

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14.0 GLOSSARY¹

Accuracy²: A measure of test performance. (a) The closeness of agreement between a test result and an accepted reference value; (b) The proportion of correct outcomes of a method. Often used interchangeably with **concordance**.

Activation (of genes): The interaction of specific molecules or molecular complexes with specific genes to initiate their expression (transcription of mRNA).

Affinity (high; low): The strength of binding of a molecule to a receptor protein.

Agonism: The binding of a substance to a receptor to initiate effects similar to those produced by the natural ligand for the receptor.

Agonist: A substance that mimics the action of an endogenous hormone.

Androgen: A class of steroid hormone, which includes testosterone and 5 α -dihydrotestosterone, responsible for the development and maintenance of the male reproductive system.

Antagonism: The binding of a substance to a receptor to inhibit or counteract the effects produced by the natural ligand for the receptor.

Antagonist: A substance that blocks or diminishes the activity of an **agonist**.

Cell-free: Not containing intact cells. May contain cell or tissue homogenates or artificial mixtures of cellular components.

^{1 1} The definitions in this Glossary are restricted to their uses with respect to endocrine mechanisms and actions.

² Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods.

Complex mixture: A mixture containing many, generally uncounted, substances, many of which are undefined (e.g., plant homogenates; fuels).

Concordance²: A measure of test performance. The proportion of all chemicals that are correctly classified as positive or negative. Often used interchangeably with **accuracy**. The concordance is highly dependent on the **prevalence** of positives in the population being examined.

C-Terminal region: The end of a protein molecule that contains a free carboxylic acid moiety.

Cytoplasm: The material inside the cell, excluding the nucleus, that contains the intracellular fluid, organelles, soluble enzymes, membrane components and other factors.

Cytosol: see Cytoplasm

Detoxification: Reduction of the toxicity (of a substance) by metabolism to a less toxic form, or by removal of the substance from the affected cell or organism.

Dextran: A viscous or semi-viscous polymer of glucose.

Dissociation constant: A measure of the ability of a molecule to be released from binding to a receptor.

DNA-regulatory activity: Refers to a DNA-binding molecule or complex that causes a change in DNA-related activities.

Domain: A region of a protein defined by its activity.

Endocrine disruption: Activity by an exogenous chemical substance that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms.

Endocrine disruptor: A substance determined to cause endocrine disruption.

Endocrine system: Made up of glands located throughout the body, the hormones that are synthesized and secreted by the glands into the bloodstream, and the receptors in the various tissues are organs that recognize and respond to the hormones.

Endogenous: Originating within the organism of interest.

Endpoint: The biological process, response, or effect assessed by a test method.

Estrogen: A class of steroid hormones, which includes 17 β -estradiol, responsible for regulation of specific female reproductive functions and for development and maintenance of the female reproductive system.

Estrogenic: Having biological activity similar to that of an estrogen.

Exogenous: Originating outside the organism of interest.

False negative²: An active substance incorrectly identified as negative by a test.

False negative rate²: The proportion of all positive (active) substances falsely identified as negative. A measure of test performance.

False positive²: An inactive substance incorrectly identified as positive by a test.

False positive rate²: The proportion of all negative (inactive) substances falsely identified as positive. A measure of test performance.

Fluorescence polarization (FP): A technique that can detect molecular interactions by monitoring changes in the polarization of fluorescently labeled or inherently fluorescent molecules.

Frog metamorphosis assay: A test method that measures the ability of a substance to affect the metamorphosis of frog larvae (tadpoles) to adults.

Gonadal recrudescence assay: A test method that measures the ability of a substance to produce effects in estrogen- and androgen-dependent accessory sex organs or gonad maturation in fish. A test method for potential estrogen- and androgen-related endocrine disruption.

Half-life: The time it takes for a chemical or radioactive substance to lose half its activity.

Hazard: An adverse health or ecological effect.

Hershberger assay: Measures the ability of a substance to alter the weight of androgen-dependent accessory sex organs (e.g., ventral prostate or seminal vesicles) or tissues in castrated rats or mice. A test method for potential androgen and anti-androgen related endocrine disruption activity.

Homology (DNA): Similarity in DNA sequence of segments or genes from different strains or species of organisms.

Hormone: A chemical substance produced in specific cells, or glands, that can either act locally or be released into the bloodstream to act on an organ or tissue in another part of the body.

Hydrophobic: Refers to chemicals and substances that will not dissolve or that sparingly dissolve in water.

Hydroxyapatite (HAP): A form of calcium phosphate with the ability to bind to some classes of organic molecules.

Hypospadias: A clinical condition in newborns that manifests itself as a displaced opening of the urethra. Occurs in males only and is considered a fetal developmental anomaly.

Inter-laboratory reproducibility²: A measure of whether different laboratories using the same protocol and test chemicals can produce qualitatively and quantitatively similar results. See **reliability**.

Intra-laboratory reproducibility²: A measure of whether the same laboratory can successfully replicate results using a specific test protocol at different times. See **reliability**.

Intraperitoneal: Administration by injection directly into the peritoneal cavity.

In vitro: In glass. Refers to assays performed in test tubes or petri dishes using single-cell organisms or under cell-free conditions.

In vivo: In the living organism. Refers to assays performed in multi-cellular organisms.

K_d: Equilibrium dissociation constant of a reference compound in a specific receptor preparation. A measure of the strength of binding between a receptor and ligand.

K_i: Equilibrium dissociation constant of an inhibitor in a competitive receptor binding experiment.

Ligand: A substance that is capable of binding to a specific receptor protein.

Ligand-binding domain: The area within a receptor molecule that attracts and holds a ligand.

Metabolic activation: Metabolism by an organism or a cell-free extract of a chemical to a biologically active form.

Negative control: An untreated sample containing all reagents of a test system, except the assay solvent, which is replaced with a known non-reactive material, such as water. This sample is processed with treated samples and other control samples to ensure the solvent does not interact with the test system.

Negative predictivity²: The proportion of correct negative responses among substances testing negative.

N-Terminal region: The end of a protein molecule that contains a free amino acid moiety.

Ovariectomized: Having the ovaries surgically removed.

Peer review: Objective review of data, a document, or proposal, and provision of recommendations, by an expert individual or group of individuals having no conflict of interest with the outcome of the review.

pH: A measure of the acidity or alkalinity of a solution. pH 7.0 is neutral; higher pHs are alkaline, lower pHs are acidic.

Placental aromatase assay: Measures the ability of a substance to induce or inhibit the activity of the aromatase enzyme, which converts testosterone to estradiol. A test method for potential anti-estrogen related endocrine activity.

Positive control: A sample containing all components of a test system and treated with a substance known to induce a positive response, that is processed with other samples to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay over time.

Positive predictivity²: The proportion of correct positive responses among substances testing positive.

Prevalence²: The proportion of positives in the population of substances tested.

Priority setting: The collection, evaluation, and analysis of existing relevant information to determine whether, and in what relative order of priority, substances will be subjected to screening or testing.

Protocol²: The precise, step-by-step description of a test, including the listing of all necessary reagents, criteria and procedures for the evaluation of the test data.

Pubertal female assay: Measures the ability of a substance to induce or inhibit the onset of puberty in an immature female rats and mice, measured as an early or late opening of the vagina. A test method for potential estrogenicity and anti-estrogenicity.

Pubertal male assay: Measures the ability of a substance to induce or inhibit prepubertal separation in immature rats and mice. At recovery (53 days), various tissues are weighed and the thyroid examined histologically. A test method for potential androgen- and anti-androgen related endocrine disruption ability.

Radiolabel: A radioactive isotope of an atom that is added to a molecule to allow the molecule to be identified by **scintillation counting**.

Receptor: A protein or protein complex, which binds to specific molecules for the purpose of transporting them elsewhere in the cell, or for producing a chemical signal.

Receptor binding assay (competitive): An assay to measure the ability of a substance to bind to a hormone receptor protein, which is typically performed by measuring the ability of the substance to displace the bound natural hormone.

Receptor superfamily: A family of related receptors with similar composition and reactivity (e.g., the estrogen, androgen, and glucocorticoid receptors).

Relevance (of an assay)²: The relationship of a test to the effect of interest and whether a test is meaningful and useful for a particular purpose. The extent to which an assay will correctly predict or measure the biological effect of interest. A measure of assay **performance**.

Reliability (of an assay)²: The intra- and inter-laboratory **reproducibility** of the assay.

Repression (of genes): The interaction of specific molecules or molecular complexes with specific genes to prevent their expression (transcription of mRNA).

Scintillation counting: The measurement of radioactivity using a scintillation counter.

Screen/Screening Test²: A rapid, simple test conducted for the purposes of a general classification of substances according to general categories of hazard. The results of a screen are generally used for preliminary decision-making and to set priorities for more definitive tests. A screening test may have a truncated response range (e.g., be able to reliably identify active chemicals but not inactive chemicals).

Sensitivity²: The proportion of all positive substances that are correctly classified as positive in a test.

Solvent control: An untreated sample containing all components of a test system, including the solvent, that is processed with treated samples and other control samples to determine whether the solvent interacts with the test system.

Specificity²: The proportion of all negative substances that are correctly classified as negative in a test.

Stereospecific: Refers to the orientation of atoms within a molecule. The specific orientation of some atoms can affect the chemical reactivity of the molecule.

Steroidogenesis assay: Measurement of the ability of chemicals to inhibit steroid hormone biosynthesis in testicular tissue or cells *in vitro*.

Sulfhydryl: Chemical containing sulfur in the form of a -SH group.

Test battery: A series of tests, usually performed at the same time or in close sequence. Each test in the battery usually measures a different component of a multi-factorial toxic effect, or a mechanistically-related effect.

Tier 1 assay for endocrine disruptors: An assay that is a component of the EDSP screening battery of tests.

Tier 1 battery for endocrine disruptors: Defined by the EDSP as a series of *in vitro* and *in vivo* tests to determine the ability of substances to interact with the endocrine system.

Tier 2 assay for endocrine disruptors: An assay that is the component of the EDSP testing battery.

Tier 2 battery for endocrine disruptors: Defined by the EDSP as a series of *in vivo* tests designed to confirm the endocrine disrupting ability of substances in laboratory animals and wildlife species.

Transcriptional activation: The initiation of mRNA synthesis in a gene in response to a specific chemical signal, such as an estrogen-estrogen receptor complex.

Transcriptional regulatory protein: A protein that binds to a specific DNA sequence resulting in a change in the regulation of mRNA synthesis.

Uterotrophic assay: Measures the ability of a substance to cause uterine enlargement in an immature or ovariectomized rat or mouse. A test method for potential estrogenicity and anti-estrogenicity.

Valid method²: A method determined to be acceptable for a specific use.

Validated method²: A method for which the reliability and relevance for a specific purpose has been established.

Validation²: The process by which the reliability and relevance of a procedure for a specific purpose are established.

Vector: A small segment of DNA (frequently a plasmid or viral DNA) that is used to carry a foreign gene or DNA sequence into a cell's nucleus.

Weight-of-evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.

Xenobiotic: A substance that is not produced by the organism of interest.

Zinc finger motif: A configuration of a DNA-binding protein that resembles a finger and binds a zinc ion for its activity.

Appendix A

***In Vitro* ER Binding Assays**

- A1 Assays Using Rat Uterine Cytosol**
- A2 Assays Using Mouse Uterine Cytosol**
- A3 Assays Using Human ER α and ER β**
- A4 Assays Using Recombinant ER α and ER β**
- A5 Assays Using Fluorescence Polarization**
- A6 Assays Using GST-ERdef Constructs**
- A7 Assays Using MCF-7 Cells and Cytosol**

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Appendix A1

Assays Using Rat Uterine Cytosol

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Assays Using Rat Uterine Cytosol

Reference	Acton et al. (1983)	Allen et al. (1980)	Anstead et al. (1989)
Preparation of receptor			
<i>Species/strain from which receptor obtained</i>	Rats (otherwise unspecified)	Rats (otherwise unspecified)	Rats (otherwise unspecified)
<i>Age of animals</i>	Mature	n.p.	n.p.
<i>Source of receptor</i>	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol
<i>When ovariectomized</i>	n.p.	estradiol benzoate treated 3 x 0.16 µg	n.p.
<i>Buffer for preparation of cytosol</i>	n.p.	TED (10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol)	n.p.
<i>Dilution of tissue with buffer</i>	n.p.	8 uteri in 4 mL TED buffer	n.p.
<i>Protein concentration of cytosol</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Volume and concentration of radiolabelled 17 β -estradiol</i>	volume n.p.; 1 nM	50 µL; 7x10 ⁻⁹ mol/L	n.p.
<i>Specific activity of radioligand</i>	n.p.	58 Ci/mmol	n.p.
<i>Solvent used to dissolve competing ligand</i>	ethanol	TED buffer	n.p.
<i>Concentration range of competing ligand</i>	10 ⁻³ to 10 µg/mL	10 ⁻⁹ to 10 ^{-5.5} mol/L	10 ⁻³ to 10 µg/mL
<i>Vol. ER prep used</i>	n.p.	150 µL	n.p.
<i>No. of replicates</i>	n.p.	n.p.	n.p.
<i>No. of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	16 hours	30 mins	n.p.
<i>Temp incubation</i>	3°C	30°C	n.p.
<i>Measure of Non-specific binding(y/n) and concentration</i>	y, 10 ⁻³ to 10 µg/mL	n.p.	n.p.
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	Dextran coated charcoal	200 µL dextran coated charcoal (250mg% Norit A, 25mg% dextran in TED buffer)	Dextran coated charcoal
<i>Incubation time and temp.</i>	n.p.	20 min. in ice cold water	n.p.
<i>Centrifugation speed</i>	n.p.	2000xg	n.p.
<i>Centrifugation time and temp</i>	n.p.	4°C for 5 min.	n.p.
Data calculations			
<i>Program or method used for calculating data</i>	n.p.	n.p.	n.p.
<i>Data plotted as</i>	n.p.	% specific ct./min. to controls vs. Conc. ligand in incubate (mol/L)	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>		graphical	n.p.
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ ligand (estimated)	IC ₅₀ E ₂ /IC ₅₀ competitor x100	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Ashby et al. (1999)	Blair et al. (2000)	Connor et al. (1997)
Preparation of receptor			
<i>Species/strain from which receptor obtained</i>	AP Rats	Sprague Dawley rats	Sprague Dawley rats
<i>Age of animals</i>	21-25 days old	245±18 days old	24 days
<i>Source of receptor</i>	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol
<i>When ovariectomized</i>	n.p.	10 days prior to sacrifice	n.p.
<i>Buffer for preparation of cytosol</i>	TEGM (10 mM Tris, 1.5 mM EDTA, MgCl ₂ 3 mM, 10% glycerol, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 10 mM D, 10% glycerol, pH 7.4)	TESHMo (10 mM Tris-HCl, pH 7.4; 1.5 mM EDTA, 15 mM thioglycerol; 10 mM sodium molybdate)
<i>Dilution of tissue with buffer</i>	50 mg/mL buffer	100 mg/mL buffer	50 mg/mL buffer
<i>Protein concentration of cytosol</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Volume and concentration of radiolabelled 17 β -estradiol</i>	volume n.p.; 5 nM - 500 μM	10 μL; 1 nM	volume n.p.; 10 nM
<i>Specific activity of radioligand</i>	n.p.	141 Ci/mmol	130 Ci/mmol
<i>Solvent used to dissolve competing ligand</i>	n.p.	100% ethanol	n.p.
<i>Concentration range of competing ligand</i>	5 nM - 500 μM	n.p.	1 mM - 0.1 μM
<i>Vol. ER prep used</i>	100 μL	50 μL	n.p.
<i>No. of replicates</i>	n.p.	2	3
<i>No. of times assay repeated</i>	n.p.	2	n.p.
<i>Time of incubation</i>	18 hours	20 hours	8 hours
<i>Temp incubation</i>	4°C	4°C	4°C
<i>Measure of Non-specific binding(y/n) and concentration</i>	n.p.	y	n.p.
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	Hydroxyapatite in TEGM buffer	750 μl cold hydroxyapatite in 50mM Tris pH 7.4	0.1 volume Dextran-coated charcoal
<i>Incubation time and temp.</i>	n.p.	20 min. at 4°C	n.p.
<i>Centrifugation speed</i>	n.p.	600xg	8000xg
<i>Centrifugation time and temp</i>	n.p.	4°C for 5 min.	10 min.; temp. n.p.
Data calculations			
<i>Program or method used for calculating data</i>	n.p.	n.p.	n.p.
<i>Data plotted as</i>	% Control vs. Concentration (M)	% [³ H]-E ₂ bound vs. Competitor concentration (M)	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	IC ₅₀	IC ₅₀
<i>Calculation of RBA</i>	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Elsby et al. (2000)	Fang et al. (2001)	Gabbard and Segaloff (1983)
Preparation of receptor			
<i>Species/strain from which receptor obtained</i>	AP rats	Sprague Dawley rats	AXC rats
<i>Age of animals</i>	21-25 days old	245±18 days old	Mature
<i>Source of receptor</i>	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol
<i>When ovariectomized</i>	n.p.	10 days prior to sacrifice	5 days prior to sacrifice
<i>Buffer for preparation of cytosol</i>	TEGM (10 mM Tris, 1.5 mM EDTA, MgCl ₂ 3 mM, 10% glycerol, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 10 mM D, 10% glycerol, pH 7.4)	1.5 mM Tris, 1.0 mM EDTA, 20 mM sodium molybdate, pH 7.4
<i>Dilution of tissue with buffer</i>	50 mg/mL buffer	17 mg/mL	10 mg/mL buffer
<i>Protein concentration of cytosol</i>	n.p.	n.p.	6 mg/mL
Competitive binding assay			
<i>Volume and concentration of radiolabelled 17 β -estradiol</i>	volume n.p.; 5 nM - 500 μM	10 μL; 1 nM	n.p.
<i>Specific activity of radioligand</i>	111 Ci/mmol	141 Ci/mmol	53 Ci/mmol
<i>Solvent used to dissolve competing ligand</i>	n.p.	100% ethanol	ethanol
<i>Concentration range of competing ligand</i>	5 nM - 500 μM	n.p.	1 nM to 1 μM
<i>Vol. ER prep used</i>	100 μL	50 μL	100 μL
<i>No. of replicates</i>	n.p.	2	3
<i>No. of times assay repeated</i>	n.p.	2	n.p.
<i>Time of incubation</i>	18 hours	20 hours	2 hours
<i>Temp incubation</i>	4°C	4°C	4°C
<i>Measure of Non-specific binding(y/n) and concentration</i>	n.p.	n.p.	n.p.
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	250 μL 60% Hydroxyapatite in TEGM buffer	750 μl cold hydroxyapatite in 50mM Tris pH 7.4	Dextran-coated charcoal (Norite-A + dextran + human gamma globulin)
<i>Incubation time and temp.</i>	n.p.	20 min. at 4°C	15 min. at 4°C
<i>Centrifugation speed</i>	1000xg	600xg	3500xg
<i>Centrifugation time and temp</i>	10 min. at room temp.	4°C for 5 min.	10 min. at 0°C
Data calculations			
<i>Program or method used for calculating data</i>	n.p.	n.p.	n.p.
<i>Data plotted as</i>	% Control vs. Concentration (M)	n.p.	% Bound radioactivity vs. Log concentration
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	RBA	relative displacing activity (RDA)
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor x100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Jaimez et al. (2000)	Jordan et al. (1986)	Laws et al. (1996)
Preparation of receptor			
<i>Species/strain from which receptor obtained</i>	Rats (otherwise unspecified)	Sprague Dawley rats	Long Evans rats
<i>Age of animals</i>	Immature	18-21 days	Adult (60 days)
<i>Source of receptor</i>	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol
<i>When ovariectomized</i>	n.p.	n.p.	11 days prior to sacrifice
<i>Buffer for preparation of cytosol</i>	TEDM (20mM Tris-HCl, 1.5 mM EDTA, 0.25 mM dithiothreitol, 10 µg/mL leupeptine, 10% glycerol, pH 7.4)	TED (10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol, pH 7.4)	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)
<i>Dilution of tissue with buffer</i>	1:6 (w/v) tissue to buffer ratio	2 uteri/mL buffer	50 mg/mL buffer
<i>Protein concentration of cytosol</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Volume and concentration of radiolabelled 17 β -estradiol</i>	volume n.p.; 1 nM	100 µL; 5x10 ⁻⁹ mol/L	volume n.p.; 1 nM
<i>Specific activity of radioligand</i>	n.p.	51 Ci/mmol	111 Ci/nmol
<i>Solvent used to dissolve competing ligand</i>	n.p.	ethanol	20% glycerol/ethanol; TE-G30%-MTG buffer
<i>Concentration range of competing ligand</i>	n.p.	n.p.	0.0001-1000 µM
<i>Vol. ER prep used</i>	n.p.	200 µL	200 µL
<i>No. of replicates</i>	n.p.	n.p.	n.p.
<i>No. of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	18 hours	18 hours or 30 min.	30 min.
<i>Temp incubation</i>	4°C	30°C or 4°C	30°C
<i>Measure of Non-specific binding(y/n) and concentration</i>	n.p.	y	y
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	Dextran-coated charcoal (250 mg Norite-A + 25 mg Dextran T-70 in 100 mL TEDM buffer)	Dextran-coated charcoal (0.25% Norit A and 0.025% dextran in TED buffer)	250 µL 60% hydroxyapatite in TEG-MTG buffer
<i>Incubation time and temp.</i>	n.p.	20 min. at 4°C	15 min. at room temp.
<i>Centrifugation speed</i>	800xg	2000xg	1000xg
<i>Centrifugation time and temp</i>	15 min. at 4°C	10 min. at 4°C	10 min. at 4°C
Data calculations			
<i>Program or method used for calculating data</i>	n.p.	n.p.	Graph Pad Prism
<i>Data plotted as</i>	n.p.	% specific [³ H]-E ₂ binding in controls vs. log concentration (mol/L)	% Specific binding vs. Competitor (M)
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	RBA	IC ₅₀	K _i calculated from EC ₅₀
<i>Calculation of RBA</i>	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor x100	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Laws et al. (2000)	Leibl and Spona (1982)	Liu et al. (1994)
Preparation of receptor			
<i>Species/strain from which receptor obtained</i>	Long Evans rats	Sprague Dawley rats	Sprague Dawley rats
<i>Age of animals</i>	Adult (60 days)	Adult (60 - 80 days)	30 days
<i>Source of receptor</i>	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol
<i>When ovariectomized</i>	11 days prior to sacrifice	10 days prior to sacrifice	n.p.
<i>Buffer for preparation of cytosol</i>	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)	TMK buffer (10 mM Tris, 1.5 mM MgCl ₂ , 10 mM KCl, pH 7.2)	TEGD (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol)
<i>Dilution of tissue with buffer</i>	50 mg/mL buffer	5 uteri/4 mL buffer	n.p.
<i>Protein concentration of cytosol</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Volume and concentration of radiolabelled 17 β -estradiol</i>	volume n.p.; 1 nM	volume n.p.; 1 nM	volume n.p.; 1 nM
<i>Specific activity of radioligand</i>	111Ci/nmol	58 Ci/mmol	147 Ci/mmol
<i>Solvent used to dissolve competing ligand</i>	20% glycerol/ethanol; TE-G30%-MTG buffer	ethanol	n.p.
<i>Concentration range of competing ligand</i>	0.0001-1000 μ M	1 pM -1 μ M	1 nM - 10 μ M
<i>Vol. ER prep used</i>	200 μ L	n.p.	200 μ g protein
<i>No. of replicates</i>	n.p.	2	n.p.
<i>No. of times assay repeated</i>	n.p.	n.p.	3
<i>Time of incubation</i>	30 min.	18 hours	2 hours
<i>Temp incubation</i>	30°C	4°C	22°C
<i>Measure of Non-specific binding(y/n) and concentration</i>	y	n.p.	y
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	250 μ L 60% hydroxyapatite in TEG-MTG buffer	0.25 mL dextran-coated charcoal (0.6% charcoal Norit A, 0.06% dextran T-60)	100 μ L Dextran coated charcoal (5% charcoal and 0.5% dextran in TEGD buffer)
<i>Incubation time and temp.</i>	15 min. at room temp.	20 min., 4°C	20 min., 4°C
<i>Centrifugation speed</i>	1000xg	3000xg	1500xg
<i>Centrifugation time and temp</i>	10 min. at 4°C	10 min.; temp. n.p.	10 min. at 4°C
Data calculations			
<i>Program or method used for calculating data</i>	Graph Pad Prism	n.p.	n.p.
<i>Data plotted as</i>	% Specific binding vs. Competitor (M)	% Bound vs. Concentration competitor	[³ H]-E ₂ complex (%) vs. ligand (nM)
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	K _i calculated from EC ₅₀	graphical	graphical (EC ₅₀ estimated)
<i>Calculation of RBA</i>	n.p.	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	McBlain (1987)	Nelson et al. (1973)	Olea et al. (1996)
Preparation of receptor			
<i>Species/strain from which receptor obtained</i>	Sprague Dawley rats	Sprague Dawley rats	Rats (otherwise unspecified)
<i>Age of animals</i>	4-5 weeks	2-5 months	Immature
<i>Source of receptor</i>	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol
<i>When ovariectomized</i>	n.p.	n.p.	n.p.
<i>Buffer for preparation of cytosol</i>	(10 mM Tris, 1.5 mM EDTA, 12 mM monothioglycerol, 10 mM sodium molybdate, 10% (v/v) glycerol, pH 7.4)	10 mM Tris-HCL+1.5 mM EDTA, pH 7.4	Phosphate buffer
<i>Dilution of tissue with buffer</i>	Uteri powdered under liquid N ₂	Uteri from 3-4 mice in 10 mL	n.p.
<i>Protein concentration of cytosol</i>	n.p.	n.p.	2 mg/mL
Competitive binding assay			
<i>Volume and concentration of radiolabelled 17 β -estradiol</i>	volume n.p.; 2 nM	volume n.p.; 2 nM	volume n.p.; 3 nM
<i>Specific activity of radioligand</i>	n.p.	48 Ci/mmol	103 Bq/mmol
<i>Solvent used to dissolve competing ligand</i>	ethanol (final conc. 1.5%)	absolute ethanol	ethanol
<i>Concentration range of competing ligand</i>	0.2 nM-20 μ M	0.1 to 500 μ M	0.1 nM to 100 μ M
<i>Vol. ER prep used</i>	n.p.	400 μ g protein	n.p.
<i>No. of replicates</i>	n.p.	n.p.	n.p.
<i>No. of times assay repeated</i>	n.p.	3	n.p.
<i>Time of incubation</i>	18 hours	1 hour	16 hours
<i>Temp incubation</i>	4°C	4°C	0-4°C
<i>Measure of Non-specific binding(y/n) and concentration</i>	y	n.p.	y
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	0.5 mL dextran-coated charcoal + 1 mg/mL BSA	0.5 mL activated charcoal and 0.5m% Dextran T40 in Tris/HCL	Dextran + charcoal
<i>Incubation time and temp.</i>	10 min.	15 min. at 4°C	n.p.
<i>Centrifugation speed</i>	12,800xg	2000xg for 5 min.	n.p.
<i>Centrifugation time and temp</i>	5 min.; temp. n.p.	5 min.; temp. n.p.	n.p.
Data calculations			
<i>Program or method used for calculating data</i>	n.p.	n.p.	n.p.
<i>Data plotted as</i>	[³ H]-E ₂ Bound (% of control) vs. Molar excess of competitor	% Inhibition of [³ H]-E ₂ binding vs. Concentration (μ M)	% Specific binding vs. Concentration (M)
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₂₅ , IC ₅₀ , K _i	graphical	graphical, RBA
<i>Calculation of RBA</i>	n.p.	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor x 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Perez et al. (1998)	Qian and Abul-Hajj (1990)	Rijks et al. (1996)
Preparation of receptor			
<i>Species/strain from which receptor obtained</i>	Rats (otherwise unspecified)	Sprague Dawley rats	Sprague Dawley rats
<i>Age of animals</i>	Immature	Immature	Mature
<i>Source of receptor</i>	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol
<i>When ovariectomized</i>	n.p.	n.p.	n.p.
<i>Buffer for preparation of cytosol</i>	Phosphate buffer	n.p.	ER buffer (10 mM Tris.HCl, 1.0 mM EDTA, 1 mM dithiothreitol, 10 mM sodium molybdate, 0.25M sucrose, pH 7.4)
<i>Dilution of tissue with buffer</i>	n.p.	n.p.	200 mg/mL buffer
<i>Protein concentration of cytosol</i>	2 mg/mL	3.5 mg/mL	3-4 mg/mL
Competitive binding assay			
<i>Volume and concentration of radiolabelled 17 β -estradiol</i>	volume n.p.; 3 nM	50 μ l; concentration n.p.	50 μ l; concentration n.p.
<i>Specific activity of radioligand</i>	103 Bq/mmol	91Ci/mmol	4.26 TBq/mmol
<i>Solvent used to dissolve competing ligand</i>	ethanol	n.p.	n.p.
<i>Concentration range of competing ligand</i>	10 pM to 100 μ M	1 nM to 3 μ M	10 pM to 2 μ M
<i>Vol. ER prep used</i>	n.p.	150 μ l	50 μ L
<i>No. of replicates</i>	n.p.	2	n.p.
<i>No. of times assay repeated</i>	n.p.	n.p.	2
<i>Time of incubation</i>	16 hours	3 hours	18 hours
<i>Temp incubation</i>	4°C	4°C	4°C
<i>Measure of Non-specific binding(y/n) and concentration</i>	y	y	n.p.
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	Dextran + charcoal	Dextran coated charcoal (10 mM Trizma base, 1.0 mM EDTA, 250 mM sucrose, 0.05% dextran, 0.5% charcoal)	n.p.
<i>Incubation time and temp.</i>	n.p.	15 min. 4°C	n.p.
<i>Centrifugation speed</i>	n.p.	2000xg	n.p.
<i>Centrifugation time and temp</i>	n.p.	0°C for 5 min.	n.p.
Data calculations			
<i>Program or method used for calculating data</i>	n.p.	n.p.	LIGAND computer program
<i>Data plotted as</i>	% Specific binding vs. Concentration (M)	n.p.	% Bound vs. Concentration competitor (nM)
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	graphical, RBA	RBA	K _i
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ competitor x 100	IC ₅₀ E ₂ /IC ₅₀ competitor x 100	K _i reference steroid/ K _i competitor x 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Routledge et al. (1998)	Waller et al. (1996)	Zacharewski et al. (1998)
Preparation of receptor			
<i>Species/strain from which receptor obtained</i>	Rats (otherwise unspecified)	Long Evans rats	Sprague Dawley rats
<i>Age of animals</i>	8-10 weeks	Adult (60 days)	22 day old
<i>Source of receptor</i>	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol
<i>When ovariectomized</i>	n.p.	11 days prior to sacrifice	n.p.
<i>Buffer for preparation of cytosol</i>	TEGM (10 mM Tris, 1.5 mM EDTA, 3 mM MgCl ₂ , 10% glycerol, pH 7.4)	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)	TEGD buffer (10 mM Tris base 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.6)
<i>Dilution of tissue with buffer</i>	50 mg/mL	50 mg/mL buffer	200 mg/mL buffer
<i>Protein concentration of cytosol</i>	n.p.	n.p.	2 mg/mL
Competitive binding assay			
<i>Volume and concentration of radiolabelled 17 β -estradiol</i>	volume n.p.; 5 nM	volume n.p.; 1 nM	30 μ L; 1 nM
<i>Specific activity of radioligand</i>	110 Ci/mmol	111Ci/nmol	84 Ci/mmol
<i>Solvent used to dissolve competing ligand</i>	n.p.	20% glycerol/ethanol; TE-G30%-MTG buffer	dimethyl sulfoxide
<i>Concentration range of competing ligand</i>	0.5 nM -500 μ M	0.0001-1000 μ M	1-1000 μ M
<i>Vol. ER prep used</i>	100 μ L	200 μ L	240 μ L
<i>No. of replicates</i>	2	n.p.	2
<i>No. of times assay repeated</i>	n.p.	n.p.	3
<i>Time of incubation</i>	18 hours	30 min.	30 min.
<i>Temp incubation</i>	4°C	30°C	30°C
<i>Measure of Non-specific binding(y/n) and concentration</i>	n.p.	y	y, 30 μ L
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	250 μ L 60% hydroxyapatite	250 μ L 60% hydroxyapatite in TEG-MTG buffer	125 μ L 60% hydroxyapatite in TEGD buffer
<i>Incubation time and temp.</i>	n.p.	15 min. at room temp.	n.p.
<i>Centrifugation speed</i>	1,000xg	1000xg	n.p.
<i>Centrifugation time and temp</i>	10 min.	10 min. at room temp.	n.p.
Data calculations			
<i>Program or method used for calculating data</i>	Ligand Competition Analysis Software (Lundon Software, Chagrin Falls, OH)	one side competitive binding curves (Graph Pad Prism)	n.p.
<i>Data plotted as</i>	% Control vs. Molarity	% Specific binding vs. Competitor (M)	[³ H]-E ₂ Bound vs. Log concentration of unlabeled competitor (M)
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	graphical	K _i calculated from EC ₅₀	IC ₅₀
<i>Calculation of RBA</i>	n.p.	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Appendix A2

Assays Using Mouse Uterine Cytosol

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Assays Using Mouse Uterine Cytosol

Reference	Chae et al. (1991)	Connor et al. (1997)	Fielden et al. (1997)	Korach et al. (1978)
Preparation of receptor				
<i>Species or cell line from which receptor obtained</i>	CD-1 (ICR) BR mice	B6C3F1 mice	CD-1 mice	CD-1 mice
<i>Age of animals/ cells</i>	8-10 weeks	24 days	Shortly after weaning	26 days
<i>Source of receptor</i>	Uterus	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol	cytosol
<i>When Ovariectomized</i>	n.p.	n.p.	n.p.	n.p.
<i>Buffer for preparation of cytosol</i>	TEGM buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl ₂ , pH 7.6)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thio-glycerol, 10mM sodium molybdate)	Ice cold TEGD buffer (10 mM tris base, 1.5 mM EDTA, 10% glycerol, 1 mM dithiothreitol, pH 7.6)	Ice cold Tris/EDTA/ glycerol
<i>Dilution of tissue with buffer</i>	50 mg tissue/mL buffer	50 mg tissue/mL buffer	50 mg tissue/ mL buffer	n.p.
<i>Protein concentration of cytosol</i>	n.p.	n.p.	2.0 mg/mL	2 mg/mL
<i>Ammonium sulfate fractionation</i>	n.a.	n.a.	n.a.	n.a.
Competitive binding assay				
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol
<i>Volume, concentration of radioligand</i>	volume n.p.; final conc 5 nM	volume n.p.; final conc 10 nM	30 µL; final conc 1 nM	volume n.p.; final conc 0.1 nM
<i>Specific activity of radioligand</i>	n.p.	130 Ci/mmol	130 Ci/mmol	110 Ci/mmol
<i>Solvent used to dissolve competing ligand</i>	n.p.	n.p.	dimethyl sulfoxide	n.p.
<i>Concentration range of competing ligand</i>	0.5 nM - 5 µM	n.p.	1 nM - 1000 µM	n.p.
<i>Volume of ER prep used</i>	100 µL	n.p.	240 µL	100 µL
<i>No. of replicates</i>	2	1	2	n.p.
<i>No. of times assay repeated</i>	3	3	n.p.	n.p.
<i>Incubation time and temp.</i>	18 hours; 4° C	8 hours; 4° C	30° C for 30 min; then cooled to 4° C	18 hours; 0-4° C
<i>Measured non-specific binding (y/n)</i>	y	n.p.	y	n.p.
Separation of ligand				
<i>Volume and Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	250 µl hydroxyapatite in TEGM	0.1 vol dextran-coated charcoal	125 µl 60% (v/v) hydroxylapatite suspension in TEGD buffer	Protamine sulfate
<i>Incubation time and temp</i>	n.p.	On ice	n.p.	10 min/4°C
<i>Centrifugation speed</i>	1,000 g	8,000 g	n.p.	2,000 g
<i>Centrifugation time and temp</i>	10 min; temp n.p.	10 min; temp n.p.	n.p.	10 min/4°C
Data calculations				
<i>Program or method used for calculating data</i>	Ligand Competition Analysis Software by EMF	n.p.		n.p.
<i>Data plotted as</i>	% receptor bound vs. molar excess competitor	No plot	Percent specific binding of [³ H]E2 vs. log concentration of competitor (M)	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	C ₅₀ and RBA	IC ₅₀	IC ₅₀	RBA
<i>Calculation of RBA</i>	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor x100	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor x100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Mouse Uterine Cytosol

Reference	Korach et al. (1979)	Korach et al. (1985)	Korach et al. (1988)	Korach et al. (1989)
Preparation of receptor				
<i>Species or cell line from which receptor obtained</i>	CD-1 mice	CD-1 (ICR) BR mice	CD-1 mice	CD-1 (ICR) BR mice
<i>Age of animals/ cells</i>	On or before 24 days	n.p.	On or before 24 days	n.p.
<i>Source of receptor</i>	Uterus	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol	cytosol
<i>When Ovariectomized</i>	5 days prior to sacrifice	7 days prior to sacrifice	5 days prior to sacrifice	7 days prior to sacrifice
<i>Buffer for preparation of cytosol</i>	Ice cold TEG buffer (10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, pH 8.0)	Ice cold TEGM buffer (pH 8.0, 4°C, 10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, 3mM MgCl ₂)	TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.6)	Ice cold TEGM buffer (pH 8.0, 4°C, 10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, 3mM MgCl ₂)
<i>Dilution of tissue with buffer</i>	n.p.	n.p.	75 mg wet weight/mL buffer	50 mg tissue/mL buffer
<i>Protein concentration of cytosol</i>	1 mg/mL approximate	1 mg/mL approximate	n.p.	1.7 mg/mL
<i>Ammonium sulfate fractionation</i>	n.a.	n.a.	cytosolic ER was enriched by a 0-40% ammonium sulfate fractionation	n.a.
Competitive binding assay				
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol
<i>Volume, concentration of radioligand</i>	volume n.p. final conc 5 nM	volume n.p.; final conc 5 nM	n.p.	volume n.p.; final conc 5 nM
<i>Specific activity of radioligand</i>	110 Ci/mmol	98 Ci/mmol	n.p.	98 Ci/mmol
<i>Solvent used to dissolve competing ligand</i>	n.p.	n.p.	n.p.	n.p.
<i>Concentration range of competing ligand</i>	1 nM - 2.5 µM	1 nM - 2.5 µM	n.p.	0.5 - 500 mM
<i>Volume of ER prep used</i>	100 µL	100 µL	n.p.	200 µL
<i>No. of replicates</i>	3	n.p.	n.p.	
<i>No. of times assay repeated</i>	5 or more	n.p.	n.p.	4
<i>Incubation time and temp.</i>	18 hours; 4° C	18 hours; 4° C	n.p.	18 hours; 4° C
<i>Measured non-specific binding (y/n)</i>	n.p.	n.p.	y	n.p.
Separation of ligand				
<i>Volume and Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	Protamine sulfate	Protamine sulfate	Hydroxylapatite adsorption	Hydroxylapatite adsorption
<i>Incubation time and temp</i>	10 min/4°C	10 min/4°C	10 min/4°C	n.p.
<i>Centrifugation speed</i>	2,000 g	2,000 g	2,000 g	n.p.
<i>Centrifugation time and temp</i>	10 min/4°C	10 min/4°C	10 min/4°C	n.p.
Data calculations				
<i>Program or method used for calculating data</i>	n.p.	n.p.	n.p.	n.p.
<i>Data plotted as</i>	Semi-log plot of % Receptor bound vs. log molar excess of unlabeled competitor	Scatchard plot (bound/unbound vs. bound)	Semi-log plot of % Receptor bound vs. log molar excess of unlabeled competitor	% receptor bound vs. molar excess competitor
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	C ₅₀ (molar equivalents of unlabeled competitor required to occupy 50% of the receptor binding sites)	C ₅₀	C ₅₀
<i>Calculation of RBA</i>	n.p.	n.p.	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Mouse Uterine Cytosol

Reference	Korach (1979)	Matthews et al. (2001)	Ramamoorthy et al. (1997a)	Ramamoorthy et al. (1997b)
Preparation of receptor				
<i>Species or cell line from which receptor obtained</i>	CD-1 mice	CD-1 mice	B6C3F1 mice	B6C3F1 mice
<i>Age of animals/ cells</i>	24 days	21 days	24 days	24 days
<i>Source of receptor</i>	Uterus	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol	cytosol	cytosol
<i>When Ovariectomized</i>	5 days prior to sacrifice	n.p.	n.p.	n.p.
<i>Buffer for preparation of cytosol</i>	Ice cold TE buffer(0.01 M Tris, .0015 M disodium EDTA, pH 8 or 7.4)	TEGD (10 nM Tris base, 1.5 mM EDTA, 10% glycerol, 1.0 mM DTT, pH 7.6)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thio-glycerol, 10mM sodium molybdate)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thio-glycerol, 10mM sodium molybdate)
<i>Dilution of tissue with buffer</i>	n.p.	50 mg tissue/ mL buffer	50 mg tissue/mL buffer	50 mg tissue/mL buffer
<i>Protein concentration of cytosol</i>	1 mg/mL approximate	2.0 mg/mL	n.p.	n.p.
<i>Ammonium sulfate fractionation</i>	n.a.	n.a.	n.a.	n.a.
Competitive binding assay				
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol
<i>Volume, concentration of radioligand</i>	volume n.p.; final conc 10 nM	5 µL; final conc 2.5 nM	n.p.	volume n.p.; final conc 10 nM
<i>Specific activity of radioligand</i>	110 Ci/mmol	n.p.	130 Ci/mmol	n.p.
<i>Solvent used to dissolve competing ligand</i>	n.p.	dimethyl sulfoxide	n.p.	n.p.
<i>Concentration range of competing ligand</i>	0.1 - 1000-fold molar excess	n.p.	10 nM - 10 µM	n.p.
<i>Volume of ER prep used</i>	100 µL	240 µL	n.p.	n.p.
<i>No. of replicates</i>	n.p.	4	n.p.	n.p.
<i>No. of times assay repeated</i>	n.p.	n.p.	n.p.	n.p.
<i>Incubation time and temp.</i>	18 hours; 4° C	2 hours; 30° C	8 hours; 4° C	16-18 hours; 4° C
<i>Measured non-specific binding (y/n)</i>	n.p.	n.p.	n.p.	n.p.
Separation of ligand				
<i>Volume and Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	Protamine sulfate	96-well filter plate and vacuum pump harvester	0.1 vol DCC suspension (0.5% dextran: 5% charcoal, wt/vol in TESHMo)	0.1 vol DCC suspension (0.5% dextran: 5% charcoal, wt/vol in TESHMo)
<i>Incubation time and temp</i>	10 min/4°C	Samples dried under suction for 30 sec; Filter plates sealed. Scintillation cocktail added to each well.	10 min; temp n.p.	10 min; temp n.p.
<i>Centrifugation speed</i>	n.p.	n.a.	5,000 g	5,000 g
<i>Centrifugation time and temp</i>	10 min/4°C	n.a.	10 min; temp n.p.	10 min; temp n.p.
Data calculations				
<i>Program or method used for calculating data</i>	n.p.	GraphPad Prism 3.0 software	n.p.	n.p.
<i>Data plotted as</i>	Scatchard plot	Percent specific binding of [³ H]E2 vs. log competitor concentration	% [³ H]E2 bound vs. -log [M]	DPM vs. log [M]
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	C ₅₀ (molar excess of unlabeled competitor which inhibits 50% specific receptor binding)	IC ₅₀	n.p.	IC ₅₀
<i>Calculation of RBA</i>	n.p.	n.p.	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Mouse Uterine Cytosol

Reference	Shelby et al. (1996)	Waller et al. (1996)
Preparation of receptor		
<i>Species or cell line from which receptor obtained</i>	CD-1 BR mice	CD-1 mice
<i>Age of animals/ cells</i>	either 8-10 weeks or 12-14 weeks	On or before 24 days
<i>Source of receptor</i>	Uterus	Uterus
<i>Isolated preparation</i>	cytosol	cytosol
<i>When Ovariectomized</i>	2 weeks prior to sacrifice	5 days prior to sacrifice
<i>Buffer for preparation of cytosol</i>	TEGM buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl ₂ , pH 7.6)	TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.6)
<i>Dilution of tissue with buffer</i>	50 mg tissue/mL buffer	75 mg wet weight/mL buffer
<i>Protein concentration of cytosol</i>	n.p.	n.p.
<i>Ammonium sulfate fractionation</i>	n.a.	cytosolic ER was enriched by a 0-40% ammonium sulfate fractionation for some binding experiments
Competitive binding assay		
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol
<i>Volume, concentration of radioligand</i>	final conc 5 nM	final conc 5 nM
<i>Specific activity of radioligand</i>	n.p.	n.p.
<i>Solvent used to dissolve competing ligand</i>	n.p.	n.p.
<i>Concentration range of competing ligand</i>	0.5 nM - 5 µM	2.5 nM - 25 µM
<i>Volume of ER prep used</i>	100 µL	100 µL
<i>No. of replicates</i>	2	n.p.
<i>No. of times assay repeated</i>	2	n.p.
<i>Incubation time and temp.</i>	18 hours; 4° C	18 hours; 4° C
<i>Measured non-specific binding (y/n)</i>	n.p.	n.p.
Separation of ligand		
<i>Volume and Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	250 µL 60% hydroxyapatite in TEGM buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl ₂ , pH 7.6)	Hydroxylapatite adsorption
<i>Incubation time and temp</i>	n.p.	10 min/4°C
<i>Centrifugation speed</i>	1,000 g	2,000 g
<i>Centrifugation time and temp</i>	10 min; temp n.p.	10 min/4°C
Data calculations		
<i>Program or method used for calculating data</i>	n.p.	n.p.
<i>Data plotted as</i>	Semi-log plot of % Receptor bound vs. Log competitor concentration (M)	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	n.p.	n.p.
<i>Calculation of RBA</i>	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Appendix A3

Assays Using Human ER α and ER β

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Assays Using Human ER α and ER β

Reference	Arcaro et al. (1999)	Arcaro et al. (2000)	Fertuck et al. (2001)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER alpha and human ER beta	human ER alpha	human ER beta
<i>Source of receptor</i>	n.p.	n.p.	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant	recombinant
<i>Buffer for isolation of receptor</i>	n.a.	n.a.	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DDT, 10% glycerol containing 1 mg/mL BSA pH 7.6)
<i>Protein concentration</i>	1.2 nM	1.2 nM	n.p.
Competitive binding assay			
<i>Radioligand used</i>	^3H -17 -estradiol	^3H -17 -estradiol	^3H -17 -estradiol
<i>Concentration of radioligand</i>	2.5 nM	2.5 nM	10 pM-1 μM
<i>Solvent used to dissolve ligand</i>	n.p.	n.p.	DMSO
<i>Concentration range of competing ligand</i>	5 nM - 100 μM	0.1 μM - 10 μM	60 nM-20 μM
<i>Number of replicates</i>	3	3	4
<i>Number of times assay repeated</i>	3	2	3
<i>Time of incubation</i>	4 hours	4 hours	24 hours
<i>Temperature of incubation</i>	room temperature	room temperature	4°C
<i>Non-specific binding measured (y/n)</i>	y	y	n.p.
Separation of ligand			
<i>Type of slurry</i>	hydroxyapatite	hydroxyapatite	n.p.
<i>Incubation time and temperature</i>	15 min; n.p.	15 min; n.p.	n.p.
<i>Centrifugation time and temperature</i>	20 min; n.p.	20 min; n.p.	n.p.
Data calculations			
<i>Program or method used for calculating data</i>	SigmaPlot	SigmaPlot	Nonlinear regression using Graphpad Prism 3.0
<i>Data plotted as</i>	% ^3H -E ₂ bound vs. log M of ligand	% ^3H -E ₂ bound vs. log M of ligand	Specific binding vs. log competitor conc.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	IC ₅₀	IC ₅₀
<i>Calculation of RBA</i>	n.p.	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ER α and ER β

Reference	Gaido et al. (1999)	Klotz et al. (1996)	Kraichely et al. (2000)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER alpha and human ER beta	human ER alpha	human ER alpha and human ER beta
<i>Source of receptor</i>	PanVera	Produced in Sf9 insect cells using a baculovirus expression system	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant	recombinant
<i>Buffer for isolation of receptor</i>	n.a.	n.p.	n.a.
<i>Protein concentration</i>	8 pmol/mL (alpha) 11 pmol/mL (beta)	0.4 nM	1.5 nM
Competitive binding assay			
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol
<i>Concentration of radioligand</i>	5 nM	2.5 nM	10 nM
<i>Solvent used to dissolve ligand</i>	n.p.	dimethyl sulfoxide or ethanol	n.p.
<i>Concentration range of competing ligand</i>	0.1 nM - 10 μ M	10 nM - 100 μ M	n.p.
<i>Number of replicates</i>	3	3	n.p.
<i>Number of times assay repeated</i>	3	2	n.p.
<i>Time of incubation</i>	overnight	1 hour	18 hours
<i>Temperature of incubation</i>	4°C	25°C	0°C
<i>Non-specific binding measured (y/n)</i>	n.p.	y	n.p.
Separation of ligand			
<i>Type of slurry</i>	hydroxyapatite	5% activated charcoal/0.5% dextran	hydroxyapatite
<i>Incubation time and temperature</i>	30 min; 4°C	10 min; 4°C	15 min; 0°C
<i>Centrifugation time and temperature</i>	10 min; n.p.	3 min; n.p.	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer
Data calculations			
<i>Program or method used for calculating data</i>	GraphPad Prism software	n.p.	n.p.
<i>Data plotted as</i>	% Binding vs. log dose (M)	% ³ H-E ₂ bound vs. [ligand] in nM	no plot of data reported
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	RBA	IC ₅₀	RBA
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ competitor	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ER α and ER β

Reference	Meyers et al. (1999)	Sun et al. (1999)	Sun et al. (1999)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER alpha and human ER beta	human ER alpha ligand binding domain	human ER beta ligand binding domain
<i>Source of receptor</i>	PanVera	expressed in E coli using pET15b vector	expressed in E coli using pET15b vector
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	truncated (amino acids 304-554)	truncated (amino acids 256-505)
<i>Buffer for isolation of receptor</i>	n.a.	50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol	50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol
<i>Protein concentration</i>	1.5 nM	n.p.	n.p.
Competitive binding assay			
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol
<i>Concentration of radioligand</i>	10 nM	10 nM	10 nM
<i>Solvent used to dissolve ligand</i>	n.p.	n.p.	n.p.
<i>Concentration range of competing ligand</i>	n.p.	n.p.	n.p.
<i>Number of replicates</i>	2	n.p.	n.p.
<i>Number of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	18 - 24 hours	18 hours	18 hours
<i>Temperature of incubation</i>	0°C	0°C	0°C
<i>Non-specific binding measured (y/n)</i>	n.p.	n.p.	n.p.
Separation of ligand			
<i>Type of slurry</i>	hydroxyapatite	hydroxylapatite	hydroxylapatite
<i>Incubation time and temperature</i>	15 min; 0°C	15 min; 0°C	15 min; 0°C
<i>Centrifugation time and temperature</i>	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer
Data calculations			
<i>Program or method used for calculating data</i>	n.p.	Ki calculated using Cheng-Prusoff equation	Ki calculated using Cheng-Prusoff equation
<i>Data plotted as</i>	no plot of data reported	no plot of data reported	no plot of data reported
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	RBA	IC ₅₀ (not reported), Ki, and RBA	IC ₅₀ (not reported), Ki, and RBA
<i>Calculation of RBA</i>	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor x100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ER α and ER β

Reference	Vakharia and Gierthy (1999)	Vakharia and Gierthy (2000)
Preparation of receptor		
<i>Species and subtype of receptor</i>	human ER alpha	human ER alpha
<i>Source of receptor</i>	PanVera	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant
<i>Buffer for isolation of receptor</i>	n.a.	n.a.
<i>Protein concentration</i>	1.2 nM	1.25 nM
Competitive binding assay		
<i>Radioligand used</i>	^3H -17 β -estradiol	^3H -17 β -estradiol
<i>Concentration of radioligand</i>	2.5 nM	2.5 nM
<i>Solvent used to dissolve ligand</i>	dimethyl sulfoxide	dimethyl sulfoxide
<i>Concentration range of competing ligand</i>	10 nM -1000 μM	50 nM - 50 μM
<i>Number of replicates</i>	3	3
<i>Number of times assay repeated</i>	n.p.	n.p.
<i>Time of incubation</i>	4 hours	4 hours
<i>Temperature of incubation</i>	room temperature	room temperature
<i>Non-specific binding measured (y/n)</i>	y	y
Separation of ligand		
<i>Type of slurry</i>	hydroxyapatite	hydroxyapatite
<i>Incubation time and temperature</i>	n.p.	n.p.
<i>Centrifugation time and temperature</i>	10 min; n.p.	10 min; n.p.
Data calculations		
<i>Program or method used for calculating data</i>	Sigmaplot software	Sigmaplot software
<i>Data plotted as</i>	% ^3H -E $_2$ bound vs. [ligand] in nM	% ^3H -E $_2$ bound vs. [ligand] in nM
<i>Data format in paper (e.g., IC$_{50}$, K$_i$)</i>	IC $_{50}$	IC $_{50}$
<i>Calculation of RBA</i>	n.a.	n.a.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Appendix A4

Assays Using Recombinant ER α and ER β

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Assays Using Recombinant ER α and ER β

Reference	Kuiper et al. (1997)	Kuiper et al. (1997)	Kuiper et al. (1998) [method a]
Preparation of receptor			
<i>Species and subtype of receptor</i>	rat ER beta	human ER alpha	human ER beta
<i>Whole, truncated, recombinant, or chimeric</i>	whole recombinant	whole recombinant	whole recombinant
<i>Method of protein synthesis</i>	in vitro using TnT-coupled reticulocyte lysate system	in vitro using TnT-coupled reticulocyte lysate system	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained
<i>RNA polymerase</i>	T7-RNA polymerase	T7-RNA polymerase	n.a.
<i>Reaction time or cell growth time</i>	90 min reaction time	90 min reaction time	48 hours cell growth time
<i>Reaction temperature</i>	30°C	30°C	n.a.
<i>Buffer for dilution of translation mixture or nuclear extract</i>	20 mM HEPES, pH 7.9; 150 mM NaCl, 10% w/v glycerol, 1 mM EDTA, 6 mM Na ₂ MoO ₄	20 mM HEPES, pH 7.9; 150 mM NaCl, 10% w/v glycerol, 1 mM EDTA, 6 mM Na ₂ MoO ₄	17 mM K ₂ HPO ₄ , 3 mM KH ₂ PO ₄ , 40 mM KCl, 6 mM monothioglycerol, pH=7.6
<i>Protein concentration</i>	10-15 pM	10-15 pM	800 pM
Competitive binding assay			
<i>Radioligand used</i>	16 -[¹²⁵ I]-estradiol	16 -[¹²⁵ I]-estradiol	³ H-17 -estradiol
<i>Concentration of radioligand</i>	125-150 pM	125-150 pM	3 nM
<i>Solvent used to dissolve ligand</i>	dimethyl sulfoxide	dimethyl sulfoxide	dimethyl sulfoxide
<i>Concentration range of competing ligand</i>	0.001 - 100 μ M	0.001 - 100 μ M	n.p.
<i>Volume of translation mixture or nuclear extract</i>	2 μ L	0.25 μ L	200 μ L nuclear extract per Scintistrip well
<i>Time to allow adhesion of ER to Scintistrip wells</i>	n.a.	n.a.	18 hours then washed 2X with buffer
<i>Temperature to allow adhesion</i>	n.a.	n.a.	ambient temperature
<i>Number of replicates</i>	2	2	n.p.
<i>Number of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	16 hours	16 hours	18 hours
<i>Temperature of incubation</i>	4°C	4°C	ambient temperature
<i>Non-specific binding measured (y/n)</i>	y	y	n.p.
Separation of ligand			
<i>Type of column</i>	Gel filtration over Sephadex G-25 column	Gel filtration over Sephadex G-25 column	Solid-phase ligand binding using Scintistrip wells
Data calculations			
<i>Program or method used for calculating data</i>	Nonlinear 4-parameter logistic model to estimate IC ₅₀ and Cheng-Prusoff equation to calculate Ki	Nonlinear 4-parameter logistic model to estimate IC ₅₀ and Cheng-Prusoff equation to calculate Ki	Nonlinear 4-parameter logistic model to estimate IC ₅₀
<i>Data plotted as</i>	% [¹²⁵ I]-E ₂ bound vs. log M of compound	% [¹²⁵ I]-E ₂ bound vs. log M of compound	no plot of data reported
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀ (not reported), Ki and RBA	IC ₅₀ (not reported), Ki and RBA	IC ₅₀ (not reported) and RBA
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor x100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Recombinant ER α and ER β

Reference	Kuiper et al. (1998) [method a]	Kuiper et al. (1998) [method b]
Preparation of receptor		
<i>Species and subtype of receptor</i>	human ER alpha	human ER beta
<i>Whole, truncated, recombinant, or chimeric</i>	whole recombinant	whole recombinant
<i>Method of protein synthesis</i>	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained
<i>RNA polymerase</i>	n.a.	n.a.
<i>Reaction time or cell growth time</i>	48 hours cell growth time	48 hours cell growth time
<i>Reaction temperature</i>	n.a.	n.a.
<i>Buffer for dilution of translation mixture or nuclear extract</i>	17 mM K ₂ HPO ₄ , 3 mM KH ₂ PO ₄ , 40 mM KCl, 6 mM monothioglycerol, pH=7.6	20 mM HEPES, pH 7.5; 150 mM KCl, 1 mM EDTA, 6mM monothioglycerol, 8.7% (v/v) glycerol
<i>Protein concentration</i>	400 pM	0.3 - 0.4 nM
Competitive binding assay		
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol
<i>Concentration of radioligand</i>	3 nM	3 nM
<i>Solvent used to dissolve ligand</i>	dimethyl sulfoxide	dimethyl sulfoxide
<i>Concentration range of competing ligand</i>	n.p.	n.p.
<i>Volume of translation mixture or nuclear extract</i>	200 μ L nuclear extract per Scintistrip well	n.p.
<i>Time to allow adhesion of ER to Scintistrip wells</i>	18 hours then washed 2X with buffer	n.a.
<i>Temperature to allow adhesion</i>	ambient temperature	n.a.
<i>Number of replicates</i>	n.p.	n.p.
<i>Number of times assay repeated</i>	n.p.	n.p.
<i>Time of incubation</i>	18 hours	18 -20 hours
<i>Temperature of incubation</i>	ambient temperature	6°C
<i>Non-specific binding measured (y/n)</i>	n.p.	n.p.
Separation of ligand		
<i>Type of column</i>	Solid-phase ligand binding using Scintistrip wells	Gel filtration over Sephadex G-25 column
Data calculations		
<i>Program or method used for calculating data</i>	Nonlinear 4-parameter logistic model to estimate IC ₅₀	Nonlinear 4-parameter logistic model to estimate IC ₅₀
<i>Data plotted as</i>	no plot of data reported	dpm bound radioligand vs. log M of compound
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀ (not reported) and RBA	IC ₅₀ (not reported) and RBA
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor x100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Recombinant ER α and ER β

Reference	Kuiper et al. (1998) [method b]	Morito et al. (2001)
Preparation of receptor		
<i>Species and subtype of receptor</i>	human ER alpha	human ER alpha from pBacPAK9/HEGO; human ER beta from pGEX-4T-2-hER
<i>Whole, truncated, recombinant, or chimeric</i>	whole recombinant	whole recombinant
<i>Method of protein synthesis</i>	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained	Sf9 cells were infected with amplified baculovirus; harvested after 72 h and a cytosolic fraction made by sonication and centrifugation of the homogenate containing the ER alpha or ER beta.
<i>RNA polymerase</i>	n.a.	n.a.
<i>Reaction time or cell growth time</i>	48 hours cell growth time	72 hours growth of cells
<i>Reaction temperature</i>	n.a.	28°C
<i>Buffer for dilution of translation mixture or nuclear extract</i>	20 mM HEPES, pH 7.5; 150 mM KCl, 1 mM EDTA, 6mM monothioglycerol, 8.7% (v/v) glycerol	40 mM Tris-HCL, pH 7.4, 0.5mM EDTA, 0.2M KCL, 10% (v/v) glycerol, 1mM dithiothreitol, 1mM PMSF
<i>Protein concentration</i>	0.3 - 0.4 nM	36 µg/mL
Competitive binding assay		
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol
<i>Concentration of radioligand</i>	3 nM	2.5 pmoles
<i>Solvent used to dissolve ligand</i>	dimethyl sulfoxide	n.p.
<i>Concentration range of competing ligand</i>	n.p.	n.p.
<i>Volume of translation mixture or nuclear extract</i>	n.p.	5 µL
<i>Time to allow adhesion of ER to Scintistrip wells</i>	n.a.	n.a.
<i>Temperature to allow adhesion</i>	n.a.	n.a.
<i>Number of replicates</i>	n.p.	n.p.
<i>Number of times assay repeated</i>	n.p.	n.p.
<i>Time of incubation</i>	18 -20 hours	16 hours
<i>Temperature of incubation</i>	6°C	0°C
<i>Non-specific binding measured (y/n)</i>	n.p.	n.p.
Separation of ligand		
<i>Type of column</i>	Gel filtration over Sephadex G-25 column	0.5% activated cgarcoal and 0.05% dextran
Data calculations		
<i>Program or method used for calculating data</i>	Nonlinear 4-parameter logistic model to estimate IC ₅₀	n.p.
<i>Data plotted as</i>	dpm bound radioligand vs. log M of compound	% ³ H E ₂ bound vs. fold excess of estradiol
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀ (not reported) and RBA	Calculated IC ₅₀ by knowing that 1 fold increase was 5nM
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor x100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

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Appendix A5

Assays Using Fluorescence Polarization

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Assays Using Fluorescent Polarization

Reference	Bolger et al. (1998)	Hanioka et al. (1999)	Hashimoto et al. (2000)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER	human ER	human ER
<i>Source of receptor</i>	PanVera	n.p.	n.p.
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant	recombinant
<i>Buffer for assay of receptor</i>	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin
<i>Protein concentration</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Ligand used</i>	ES2	ES2	ES2
<i>Concentration of estrogen</i>	1 nM	1 nM	1 nM
<i>Fluorescent ligand</i>	FES1 ER 13 nM, ER 10 nM	FES1 ER 13 nM, ER 10 nM	FES1 ER 13 nM, ER 10 nM
<i>Concentration of Fluorescent ligand</i>	2 nM	2 nM	2 nM
<i>Solvent used to dissolve competing ligand</i>	10 mM ethanol	10 mM ethanol	10 mM ethanol
<i>Concentration range of competing ligand</i>	200 µM	200 µM	200 µM
<i>Number of replicates</i>	3	3	3
<i>Number of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	60 min	60 min	60 min
<i>Temperature of incubation</i>	room temp	room temp	room temp
Data calculations			
<i>Fluorescence anisotropy</i>	490 nm excitation; 530 nm emission filter	360 nm excitation; 535 nm emission filter	360 nm excitation; 530 nm emission filter
<i>Program or method used for calculating data</i>	Anisotropy converted to fraction bound	Non-linear least squares regression	Anisotropy converted to percent inhibition
<i>Data plotted as</i>	Ligand bound=fraction bound x ligand conc.	Millipolarization vs. conc. Chemicals	Percent inhibition vs. competitor conc.
<i>Data format in paper (e.g., IC_{50}, K_i)</i>	K_d	IC_{50}	Percent inhibition
<i>Calculation of RBA</i>	Non-linear least squares regression	Non-linear least squares regression	n.a.

Abbreviations: n.p. = not provided;
n.a. = not applicable; RBA = relative
binding affinity

Assays Using Fluorescent Polarization

Reference	Nikov et al. (2000)	Nikov et al. (2001)	Parker et al. (2000)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER α and ER β	human ER α and ER β	human ER α and ER β
<i>Source of receptor</i>	PanVera	PanVera	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant	n.p.
<i>Buffer for assay of receptor</i>	100 mM K ₂ PO ₄ pH 7.5; 100 μ g/ml bovine gamma globulin, 0.02% sodium azide	100 mM K ₂ PO ₄ pH 7.5; 100 μ g/ml bovine gamma globulin, 0.02% sodium azide	100 mM K ₂ PO ₄ pH 7.5; 100 μ g/ml bovine gamma globulin, 0.02% sodium azide
<i>Protein concentration</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Ligand used</i>	ES2	ES2	ES2
<i>Concentration of estrogen</i>	1 nM	1 nM	1 nM
<i>Fluorescent ligand</i>	FES1	FES1	FES1 ER α 13 nM, ER β 10 nM
<i>Concentration of Fluorescent ligand</i>	n.p.	n.p.	n.p.
<i>Solvent used to dissolve competing ligand</i>	8 mM ethanol	8 mM ethanol	n.p.
<i>Concentration range of competing ligand</i>	n.p.	n.p.	n.p.
<i>Number of replicates</i>	n.p.	n.p.	n.p.
<i>Number of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	60 min	60 min	2 hours
<i>Temperature of incubation</i>	room temp	room temp	room temp
Data calculations			
<i>Fluorescence anisotropy</i>	490 nm excitation; 530 nm emission filter	490 nm excitation; 530 nm emission filter	483 nm excitation; 536 nm emission filter
<i>Program or method used for calculating data</i>	Non-linear least squares regression, Prism, Graphpad (San Diego, CA)	Non-linear binding isotherm	Non-linear least squares regression, Prism, Graphpad (San Diego, CA)
<i>Data plotted as</i>	Percent inhibition vs. competitor conc.	Percent inhibition vs. competitor conc.	Millipolarization vs. conc. Chemicals
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	IC ₅₀	IC ₅₀
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ ligand X100	IC ₅₀ E ₂ /IC ₅₀ ligand X100	IC ₅₀ E ₂ /IC ₅₀ ligand X100

Abbreviations: n.p. = not provided;
n.a. = not applicable; RBA = relative
binding affinity

Assays Using Fluorescent Polarization

Reference	Saito et al. (2000)
Preparation of receptor	
<i>Species and subtype of receptor</i>	human ER
<i>Source of receptor</i>	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant
<i>Buffer for assay of receptor</i>	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin
<i>Protein concentration</i>	n.p.
Competitive binding assay	
<i>Ligand used</i>	ES2
<i>Concentration of estrogen</i>	1 nM
<i>Fluorescent ligand</i>	FES1 ER 13 nM, ER 10 nM
<i>Concentration of Fluorescent ligand</i>	2 nM
<i>Solvent used to dissolve competing ligand</i>	10 mM ethanol
<i>Concentration range of competing ligand</i>	10 nM -10 µM
<i>Number of replicates</i>	3
<i>Number of times assay repeated</i>	n.p.
<i>Time of incubation</i>	60 min
<i>Temperature of incubation</i>	room temp
Data calculations	
<i>Fluorescence anisotropy</i>	490 nm excitation; 530 nm emission filter
<i>Program or method used for calculating data</i>	n.p.
<i>Data plotted as</i>	Percent inhibition vs. competitor conc.
<i>Data format in paper (e.g., IC_{50}, K_i)</i>	n.p.
<i>Calculation of RBA</i>	n.p.

Abbreviations: n.p. = not provided;
n.a. = not applicable; RBA = relative
binding affinity

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Appendix A6

Assays Using GST-ERdef Constructs

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Assays Using GST-ERdef Constructs

Reference	Fertuck et al. (2001)	Matthews and Zacharewski (2001)	Matthews et al. (2000)
Preparation of receptor			
<i>Species and subtype of receptor</i>	GST-hER	GST-hER def, -aERdef, -cERdef, -rtERdef	GST-hER def, -aERdef, -cERdef, -rtERdef
<i>Whole, truncated, recombinant, or chimeric</i>	Recombinant, truncated	Recombinant, truncated fusion protein	Recombinant, truncated fusion protein
<i>cDNA contained in</i>	pGEX-hER def	pGEX-ERdef	pGEX-ERdef
<i>Buffer for dilution of receptor</i>	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)
<i>Protein concentration</i>	1 mg/mL	1 mg/mL	1 mg/mL
Competitive binding assay			
<i>Radioligand used and volume</i>	5 μ L of $^3\text{H-E}_2$	5 μ L of $^3\text{H-E}_2$	5 μ L of $^3\text{H-E}_2$
<i>Concentration of radioligand</i>	2.5 nM	0.1-3.5 nM	0.1-3.5 nM
<i>Solvent used to dissolve ligand</i>	DMSO	5 μ L DMSO	5 μ L DMSO
<i>Concentration range of competing ligand</i>	60 nM-20 μ M	1 nM-10 μ M	1 nM-10 μ M
<i>Volume of receptor</i>	240 μ L	240 μ L	240 μ L
<i>Number of replicates</i>	4	4	4
<i>Number of times assay repeated</i>	3	n.p.	n.p.
<i>Time of incubation</i>	2 hours	2 hours	2 hours
<i>Temperature of incubation</i>	4°C	4°C	4°C
<i>Non-specific binding measured (y/n)</i>	n.p.	y, 400x excess E_2	y, 400x excess E_2
Separation of ligand			
<i>Type of column</i>	n.p.	96-well filter plate and harvester of bound radioligand	96-well filter plate and harvester of bound radioligand
<i>Washing solution</i>	n.p.	TEG buffer (10mMTris, pH 7.6 1.5mM EDTA, 1mM DDT, 10% glycerol containing 1mg/mL BSA)	TEG buffer (10mMTris, pH 7.6 1.5mM EDTA, 1mM DDT, 10% glycerol containing 1mg/mL BSA)
Data calculations			
<i>Program or method used for calculating data</i>	Nonlinear regression	Nonlinear regression using Graphpad Prism 3.0	Nonlinear regression using Graphpad Prism 3.0
<i>Data plotted as</i>	Specific binding vs. log competitor conc.	Percent specific binding vs. log competitor conc.	Percent specific binding vs. log competitor conc.
<i>Data format in paper (e.g., IC_{50}, K_i)</i>	IC_{50}	IC_{50}	IC_{50}
<i>Calculation of RBA</i>	$\text{IC}_{50} \text{ E}_2/\text{IC}_{50} \text{ ligand}$	$\text{IC}_{50} \text{ E}_2/\text{IC}_{50} \text{ ligand}$	$\text{IC}_{50} \text{ E}_2/\text{IC}_{50} \text{ ligand}$

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

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Appendix A7

Assays Using MCF-7 Cells and Cytosol

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Assays Using MCF-7 Cells and Cytosol

Reference	Arcaro et al. (1999)	Brooks et al. (1987)	Brooks et al. (1987)
Preparation of receptor			
<i>Species or cell line</i>	MCF-7 cells	MCF-7 cells	MCF-7 cells
<i>Whole cells/ cell homogenate</i>	whole cells	homogenate	whole cells
<i>Serum source</i>	5% calf serum	10% calf serum	n.p.
<i>Serum stripping method</i>	n.p.	n.p.	n.p.
<i>Residual E₂ in serum</i>	n.p.	n.p.	n.p.
<i>No. treated cells</i>	n.p.	n.p.	n.p.
<i>Buffer for preparation of cell homogenate or cytosol</i>	n.p.	Tris-EDTA + reducing agent, pH 7.4	n.p.
<i>Protein concentration of cytosol</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Vol and Conc. of ³H-estradiol</i>	0.1 nM	n.p.	3 nM
<i>Specific activity of labelled E₂</i>	140-150 Ci/mmol	n.p.	n.p.
<i>Test chemical solvent</i>	DMSO	n.p.	n.p.
<i>Concentration range of competing ligand</i>	5, 1, 0.5 μ M	n.p.	n.p.
<i>No. replicates</i>	quadruplicate	n.p.	n.p.
<i>Time of incubation</i>	3 hours	n.p.	1 hour
<i>Temp incubation</i>	37°C	n.p.	37°C
<i>Measure of non-specific binding</i>	n.p.	n.p.	n.p.
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	ethanol in PBS	charcoal/dextran in Tris-EDTA, pH 7.4	ethanol
<i>Incubation time and temp</i>	n.p.	n.p.	n.p.
<i>Centrifugation speed</i>	n.p.	1000g	n.p.
<i>Centrifugation time and temp</i>	n.p.	10 min, 4°C	n.p.
<i>Resuspension volume and buffer for pellet</i>	200 μ L	n.p.	n.p.
<i>Extraction of label</i>	ethanol	n.p.	ethanol
Data calculations			
<i>Program or method used to calculate data</i>	SigmaPlot	n.p.	n.p.
<i>Data plotted as</i>	linear regression	n.p.	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	% displacement of E ₂	n.p.	n.p.
<i>Calculation of RBA</i>	from IC ₅₀	Scatchard plot	Scatchard plot

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using MCF-7 Cells and Cytosol

Reference	Dodge et al. (1996)	Kramer et al. (1997)	Lascombe et al. (2000)
Preparation of receptor			
<i>Species or cell line</i>	MCF-7 cells	MCF-7 cells	MCF-7 cells
<i>Whole cells/ cell homogenate</i>	cell lysate	cytosol	whole cells
<i>Serum source</i>	10% fetal bovine serum	10% fetal bovine serum	0.1% bovine serum
<i>Serum stripping method</i>	dextran/charcoal	dextran/charcoal	dextran/charcoal
<i>Residual E₂ in serum</i>	n.p.	5 pg/ml (18 pm)	n.p.
<i>No. treated cells</i>	n.p.	n.p.	monolayer culture
<i>Buffer for preparation of cell homogenate or cytosol</i>	n.p.	Tris-EDTA-DTT-molybdate, pH 7.5; 4°C	n.p.
<i>Protein concentration of cytosol</i>	0.5 mg/ml	n.p.	n.p.
Competitive binding assay			
<i>Vol and Conc. of ³H-estradiol</i>	0.5 nM	10 nM	0.1 nM
<i>Specific activity of labelled E2</i>	n.p.	n.p.	n.p.
<i>Test chemical solvent</i>	n.p.	ethanol	ethanol
<i>Concentration range of competing ligand</i>	0.00001- 1 µM	70-0.01 µM	n.p.
<i>No. replicates</i>	n.p.	duplicate	quadruplicate
<i>Time of incubation</i>	18 hours	2 hours	1 hour
<i>Temp incubation</i>	4°C	4°C	37°C
<i>Measure of non-specific binding</i>	n.p.	n.p.	n.p.
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	charcoal/dextran; 0.07 ml	hydroxyapatite	ethanol in phosphate buffer, pH 7.4
<i>Incubation time and temp</i>	n.p.	n.p.	n.p.
<i>Centrifugation speed</i>	n.p.	800g	n.p.
<i>Centrifugation time and temp</i>	n.p.	10 min, 4°C	n.p.
<i>Resuspension volume and buffer for pellet</i>	n.p.	n.p.	n.p.
<i>Extraction of label</i>	n.p.	n.p.	ethanol
Data calculations			
<i>Program or method used to calculate data</i>	n.p.	n.p.	Student's t-test
<i>Data plotted as</i>	n.p.	nonlinear regression	% control vs. molar excess of competitor
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	DPM/nM	log IC ₅₀	bound E ₂ vs molar excess
<i>Calculation of RBA</i>	from IC ₅₀	Scatchard plot	% control

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using MCF-7 Cells and Cytosol

Reference	Miodini et al. (1999)	Nagel et al. (1997)	Palomino et al. (1994)
Preparation of receptor			
<i>Species or cell line</i>	MCF-7 cells	MCF-7 cells	MCF-7 cells
<i>Whole cells/ cell homogenate</i>	homogenate	whole cells	cytosol
<i>Serum source</i>	2% fetal calf	calf serum	n.p.
<i>Serum stripping method</i>	n.p.	charcoal	n.p.
<i>Residual E₂ in serum</i>	n.p.	n.p.	n.p.
<i>No. treated cells</i>	n.p.	n.p.	n.p.
<i>Buffer for preparation of cell homogenate or cytosol</i>	K ₂ HPO ₄ -EDTA, glycerol, thioglycerol, pH 7.4	n.p.	n.p.
<i>Protein concentration of cytosol</i>	n.p.	n.p.	n.p.
Competitive binding assay			
<i>Vol and Conc. of ³H-estradiol</i>	5 nM (16 -I-estradiol)	1 nM	1.5 nM
<i>Specific activity of labelled E2</i>	8150 GBq/mM	104 Ci/mol	100 Ci/mmol
<i>Test chemical solvent</i>	n.p.	ethanol	n.p.
<i>Concentration range of competing ligand</i>	0.0025 - 25 µM	0.1-100 µM	1.5 - 3,000 nM
<i>No. replicates</i>	quadruplicate	n.p.	triplicate
<i>Time of incubation</i>	overnight	18 hours	overnight
<i>Temp incubation</i>	4°C	37°C	4°C
<i>Measure of non-specific binding</i>	n.p.	n.p.	n.p.
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	n.p.	HBSS/BSA	charcoal/dextran
<i>Incubation time and temp</i>	n.p.	n.p.	n.p.
<i>Centrifugation speed</i>	n.p.	n.p.	n.p.
<i>Centrifugation time and temp</i>	n.p.	n.p.	n.p.
<i>Resuspension volume and buffer for pellet</i>	n.p.	1 mL	n.p.
<i>Extraction of label</i>	n.p.	HBSS	n.p.
Data calculations			
<i>Program or method used to calculate data</i>	Latin Square	n.p.	n.p.
<i>Data plotted as</i>	n.p.	n.p.	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	% binding	% inhibition vs M	n.p.
<i>Calculation of RBA</i>	n.p.	RBA	Scatchard plot

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using MCF-7 Cells and Cytosol

Reference	Rijks et al. (1996)	Soto et al. (1995)	Stoessel and Leclercq (1986)
Preparation of receptor			
<i>Species or cell line</i>	MCF-7 cells	MCF-7 cells	MCF-7 cells
<i>Whole cells/ cell homogenate</i>	homogenate	cytosol	whole cells
<i>Serum source</i>	n.p.	plasma-derived human	fetal calf serum
<i>Serum stripping method</i>	n.p.	dextran/charcoal	
<i>Residual E₂ in serum</i>	n.p.	<0.01 pg/ml	
<i>No. treated cells</i>		n.p.	monolayer culture
<i>Buffer for preparation of cell homogenate or cytosol</i>	Tris-EDTA-DTT-molybdate, pH 7.4; 4°C	KCl-EDTA-Tris, pH 7.4	n.p.
<i>Protein concentration of cytosol</i>	1.4 mg/ml	n.p.	n.p.
Competitive binding assay			
<i>Vol and Conc. of ³H-estradiol</i>	4.8x10 ⁻⁹	2 nM	1 nM
<i>Specific activity of labelled E2</i>	4.26 TBq/mmol	n.p.	100 Ci/mmol
<i>Test chemical solvent</i>	n.p.	DMSO or ethanol	ethanol
<i>Concentration range of competing ligand</i>	1x10 ⁻¹¹ - 2x10 ⁻⁶	1 pM - 1 mM	0.1 nM - 10 µM
<i>No. replicates</i>	duplicate	n.p.	triplicate
<i>Time of incubation</i>	18 hours	16 hours	50 min
<i>Temp incubation</i>	0-4°C	4°C	37°C
<i>Measure of non-specific binding</i>	n.p.	n.p.	n.p.
Separation of ligand			
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	charcoal/dextran in SHBG	charcoal/dextran	n.p.
<i>Incubation time and temp</i>	4°C	n.p.	n.p.
<i>Centrifugation speed</i>	800g	n.p.	n.p.
<i>Centrifugation time and temp</i>	7 min; 4°C	n.p.	n.p.
<i>Resuspension volume and buffer for pellet</i>	n.p.	n.p.	n.p.
<i>Extraction of label</i>	n.p.	n.p.	n.p.
Data calculations			
<i>Program or method used to calculate data</i>	n.p.	n.p.	n.p.
<i>Data plotted as</i>	n.p.	n.p.	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	n.p.	n.p.	RBA
<i>Calculation of RBA</i>	n.p.	n.p.	IC ₅₀ E ₂ /IC ₅₀ test compound X 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using MCF-7 Cells and Cytosol

Reference	VanderKuur et al. (1993)
Preparation of receptor	
<i>Species or cell line</i>	MCF-7 cells
<i>Whole cells/ cell homogenate</i>	whole cells
<i>Serum source</i>	5% calf serum
<i>Serum stripping method</i>	none
<i>Residual E_2 in serum</i>	none
<i>No. treated cells</i>	n.p.
<i>Buffer for preparation of cell homogenate or cytosol</i>	n.p.
<i>Protein concentration of cytosol</i>	n.p.
Competitive binding assay	
<i>Vol and Conc. of 3H-estradiol</i>	n.p.
<i>Specific activity of labelled E_2</i>	n.p.
<i>Test chemical solvent</i>	n.p.
<i>Concentration range of competing ligand</i>	n.p.
<i>No. replicates</i>	n.p.
<i>Time of incubation</i>	n.p.
<i>Temp incubation</i>	4°C
<i>Measure of non-specific binding</i>	n.p.
Separation of ligand	
<i>Type of slurry (Hydroxyapatite, charcoal, protamine sulfate)</i>	charcoal/dextran
<i>Incubation time and temp</i>	n.p.
<i>Centrifugation speed</i>	n.p.
<i>Centrifugation time and temp</i>	n.p.
<i>Resuspension volume and buffer for pellet</i>	n.p.
<i>Extraction of label</i>	n.p.
Data calculations	
<i>Program or method used to calculate data</i>	n.p.
<i>Data plotted as</i>	n.p.
<i>Data format in paper (e.g., IC_{50}, K_i)</i>	n.p.
<i>Calculation of RBA</i>	Scatchard plot

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

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Appendix B

***In Vitro* ER Binding Assay Protocols**

- B1 Protocol for the Competitive ER binding MCF-7 (Whole Cell Assay)**
(Provided by Dr. Guy Leclercq, Clinique et Laboratoire de Cancerologie
Mammaire, Centre des Tumeurs de l'Université Libre de Bruxelles,
Brussels, Belgium)
- B2 Protocol for the Fluorescence Polarization Assay of the Competitive
Binding of Ligands to Estrogen-Receptor Complexes**
(Provided by Dr. William Allworth, Department of Chemistry, University
of New Orleans, New Orleans, LA, USA)
- B3 Protocol for the Competitive Ligand Binding Assay**
(Provided by Dr. Timothy Zacharewski, Dept. of Biochemistry, Michigan
State University, Lansing, MI, USA)
- B4 Standard Operating Procedure for the Rat Estrogen Receptor
Equilibrium Exchange Assay**
(Provided by Dr. Weida Tong, Division of Genetic and Reproductive
Toxicology, National Center for Toxicological Research, Jefferson, AR,
USA)
- B5 Protocol for the Estrogen Receptor Competitive Binding Assay Using Rat
Uterine Cytosol**
(Provided by Dr. Susan Laws, U.S. EPA, NHEERL, Research Triangle
Park, NC, USA and Mr. Gary Timm, U.S. EPA, Washington, DC, USA)

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Appendix B1

Protocol for the Competitive ER binding MCF-7 (Whole Cell Assay)

**(Provided by Dr. Guy Leclercq, Clinique et Laboratoire de
Cancerologie Mammaire, Centre des Tumeurs de l'Universite
Libre de Bruxelles, Brussels, Belgium)**

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Stoessel and Leclercq, J Steroid Biochemistry **25**(5A):677-682, 1986.

PROTOCOL FOR THE COMPETITIVE ER BINDING

(Whole Cell Assay)

1. Culture conditions : MCF-7 cells are maintained at 37°C in a 5% CO₂ atmosphere in phenol red-free minimal essential medium (MEM) containing 10% charcoal stripped calf serum and penicillin, streptomycin, glutamine.
2. MCF-7 cells (20,000 cells/ml) are incubated for 4 days in 24 multiwells (NUNC) under above mentioned conditions.
3. After 4 days of culture, the medium is removed and the cells are incubated for 1 hour at 37° C with 1 nM [³H]estradiol (E₂) or investigated compound (X) at concentrations ranging from 1 nM to 1µM.
4. Medium is again removed and the cells washed twice with phosphate buffer saline (PBS).
5. 250 µl absolute ethanol are added to each well (exposition during 20 min).
6. Aliquots of 200 µl of supernatant (ethanol extract) are added to 3.8 ml of scintillation liquid for radioactivity measurements (10 min, counting).
7. RBA data are established from the mean of 3 independent experiments, each performed in triplicate.
8. Relative binding affinity :
$$RBA = \frac{(I_{50})E_2}{(I_{50})x} \times 100$$

I₅₀ = concentration producing 50% inhibition of [³H]E₂ incorporation (dpm of ethanol extracts).

Brussels, December 2001.

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Appendix B2

Protocol for the Fluorescence Polarization Assay of the Competitive Binding of Ligands to Estrogen-Receptor Complexes

**(Provided by Dr. William Allworth, Department of Chemistry,
University of New Orleans, New Orleans, LA, USA)**

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Fluorescence Polarization Assay of the Competitive Binding of Ligands to Estrogen-Receptor Complexes

Purified expressed human estrogen receptor alpha (ER) and human estrogen receptor beta (ER), fluorescent-labeled 17 -estradiol (ES2), and estrogen screening buffer were purchased from Pan Vera Corporation.

A solution of ER-ES2 complex in estrogen screening buffer containing 20 nM ES2, 26 nM human-ER and 10 mM dithiothreitol (DTT) was prepared, and 50 μ l of this solution was added to borosilicate test tubes containing 50 μ l of serially diluted compounds to be tested as potential estrogenic ligands. Samples tubes containing 50 μ l of the estrogen screening buffer and 50 μ l of the ER-ES2 complex solution were included as negative controls to determine the initial polarization value (P_0 , polarization value with no estrogen competitor present). Sample tubes containing 100 μ l of the estrogen screening buffer with 10 nM ES2 and 5 mM DTT were also included to determine the polarization value of nonbound ES2 (P_{100}). The samples were incubated at room temperature for 1 hour and the fluorescence polarization (FP) then measured using a Beacon 2000 Fluorescence Polarization Instrument (Pan Vera) equipped with a 530 nm emission filter and a 490 nm excitation filter.

FP values were converted to percentage inhibition using the equation

$$I_{\%} = (P_0 - P) / (P_0 - P_{100}) \times 100$$

where P_0 is the FP value at 0%, P_{100} is the FP value when 100% of the ES2 has been competitively displaced from the ER complex, and P is the experimental FP value at each concentration of the competing ligand being tested. The percentage inhibition versus competitor concentration curves were analyzed by nonlinear least-squares curve fitting and the concentration of competing ligand required to displace half of the bound ES2 fluorescent ligand determined (IC_{50}). The IC_{50} values were converted to relative binding affinities (RBA) using 17 -estradiol (E_2) as a standard. The RBA for E_2 was set equal to 100 and the RBA value for each competing ligand calculated using the following formula:

$$RBA = (IC_{50} E_2 / IC_{50} \text{ competitor}) \times 100 .$$

Measuring Estrogen Receptor-Estrogen Response Element Binding by Fluorescence Polarization

Preparation of fluorescence labeled estrogen response elements.

Sense and antisense oligonucleotide strands 35 bases long containing either estrogen response elements (EREs) from the Xenopus vit A2 gene or the human pS2 gene, or the consensus glucocorticoid response element (negative control) were obtained from Oligos Etc. (Wilsonville, OR). The sense DNA strands were labeled with fluorescein attached via a six-carbon spacer at the 5' terminus. Double stranded oligonucleotides were then prepared by annealing equimolar concentrations of the separate sense and antisense strands in 10 mM Tris-HCl, pH 7.8, and 1.50

mM NaCl as follows: 1 nmole of the sense and 1 nmole of the antisense DNA strands in 500 μ L buffer were heated in water bath to 95°C for 10 min and slowly cooled (30 min) to room temperature. To remove any hairpin formations the double stranded DNA was purified by electrophoresis on 12% polyacrylamide (1:19 bisacrylamide:acrylamide) gels containing 89 mM Tris-borate, 2.5 mM EDTA, pH 8.3, and 10% ammonium persulphate.

ER-ERE binding studies.

The abilities of ligand bound ER and ER to associate with Xenopus vit A2 ERE or human pS2 ERE were measured using fluorescence polarization (FP). Purified, expressed human ER and ER obtained from Pan Vera Corporation were serially diluted from 450 nM to 0.8 nM in DNA binding buffer (10 mM potassium phosphate, pH7.8; 0.1 mM EDTA; 50 μ M magnesium chloride; 10% glycerol). The concentrations of the ligands required to saturate ER or ER were determined by FP competitive binding experiments. Each ER was then incubated with saturating levels of the individual estrogenic ligands for 30 min, and then for 10 min with poly (dI-dC) (1 μ g/5 μ g of protein) at room temperature. The binding, initiated by adding fluorescein-labeled synthetic oligonucleotide EREs (final concentration 0.5 nM), was allowed to proceed at room temperature for 60 min in dark. The same experiment was performed with the ERs bound to 17 β -estradiol (E₂) as positive controls. The samples were prepared and measured in borosilicate test tubes with final reaction volume of 100 μ l. The FP at each ER concentration was measured on Beacon 2000 Fluorescence Polarization Instrument (Pan Vera Corporation) equipped with 490 nm excitation and 530 nm emission filters. Binding isotherms were constructed by plotting percent saturation versus ER concentration using the formula:

$$S\% = (P - P_0) / (P_{100} - P_0) \times 100$$

where P₀ is the polarization value at 0% saturation, P₁₀₀ is the polarization value at 100% saturation, and P is the observed fluorescence polarization (FP) at each concentration point. The equilibrium dissociation constant (K_d) was calculated from the binding curves using a nonlinear least-squares curve fitting program. To compare the binding affinities of ER-ligand and ER-ligand complexes for the various EREs the K_d values were converted to relative binding affinities (RBA) using the following formula with the E₂-ER complex as the standard.

$$RBA = (K_d E_2 / K_d \text{ competitor}) \times 100.$$

Appendix B3

Protocol for the Competitive Ligand Binding Assay

**(Provided by Dr. Timothy Zacharewski, Dept. of Biochemistry,
Michigan State University, Lansing, MI, USA)**

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Competitive Ligand Binding Assay

1. Caution: this protocol requires the use of radioactivity. Proper handling and disposal of all radioactive samples should be followed as outlined by the institution's safety office.
2. Prepare TEGD buffer by adding DTT to a final concentration of 1 mM to TEG buffer.
3. Add BSA (carrier protein) to a final concentration of 1 mg/ml.
4. Thaw receptor on ice. Using the appropriate dilution factor, add the receptor. As a rule of thumb, ~25 ml of TEGD+receptor is required per 96-well plate (240 μ l per tube). Keep on ice until adding to tubes in step 7.
5. Label rack of 96 1 ml glass test tubes (Marsh Scientific).
6. Add 5 μ l of radiolabeled compound at appropriate concentration to each tube using 8-channel pipettor.
7. Pipet 5 μ l of unlabeled competitor into each tube. A typical assay may involve 5 concentrations of competitor plus solvent alone, with each concentration being run in quadruplicate. For example: A1-D1 are DMSO, A2-D2 have compound A at 10^{-10} M, A3-D3 have compound A at 10^{-9} M, ... and A6-D6 have compound A at 10^{-6} M. A similar scheme is set up for compound B (A7-D12), compound C (E1-H6), and compound D (E7-H12). Typically, compound A is the 'cold' version of the radiolabeled compound.
8. Transfer 240 μ l of TEGD+receptor to each tube, using 8-channel pipettor.
9. Label and place an empty test tube rack on ice. Vortex each test tube from step 8 and place in new rack, ensuring that the order of tubes remains unchanged.
10. Place cover on glass tube rack and incubate at 4°C for 24 hrs.
11. At time of harvest, fill head of harvester with millipore-filtered water and place the head of the harvester (Packard Filtermate 196) into position.
12. Put the positioning bracket in place. Invert the 'wash' filter plate and place it within the bracket so position A12 is now in the top left. Gently close the harvester unit by pulling down the lever.
13. Turn the pump on.
14. Push and lock the 'cold' vacuum circuit on the harvester. Placing the collection tray against the intake vacuum inlets and wash for 30 sec.
15. Dry the wash plate by opening the harvester and applying continued suction for 10 sec.
16. Place a new filter plate in the harvester. Rinse the plate once with 50 ml of cold TEG buffer using the 'cold' circuit.
17. Switch to 'hot' circuit. Place the rack of 96 test tubes underneath the head unit and raise into place until all the liquid has passed through the harvester.
18. Wash with 3 x 50 ml of cold TEG buffer, leaving harvester on 'hot' circuit for all three washes. Dry as in step 15.
19. Wash harvester as in steps 11-14.
20. Label and date the filter plate. Place in radioactive hood for 10 min.
21. Put back seal on each plate and add 50 μ l of Microscint20 (Packard) to each well. Remember that the plate is now inverted (tube A1 is now bound to filter location A12).
22. Put top seal on plate and incubate at room temperature at least 30 min. Count the plate using the Receptor Binding Protocol on the TopCount Scintillation counter (Packard).

TEG Buffer

10 mM Tris
1.5 mM EDTA
10% Glycerol
pH 7.6

GST Purification

Bacterial overexpression

1. Transform *E. coli* BL 21 cells with appropriate pGEX vector. Allow colonies to grow all day.
2. Pick 2-3 colonies at the end of the day and allow them to grow overnight in 3 ml LB-Amp.
3. Perform miniprep. Add more LB-Amp (~2 ml) to starter cultures and place in shaker incubator all day (6-8 hr) at 37°C and 225 rpm.
4. Check miniprep using appropriate restriction enzymes. Select a single colony for overexpression.
5. Inoculate 50 ml of LB-Amp with 500 µl of starter culture. Incubate overnight at 37°C and 225rpm.
6. Inoculate 500 ml of LB-Amp with 5 ml of culture. (Often ~6L (i.e. 12 flasks) are inoculated.) Grow at 37°C and 225 rpm.
7. Induce culture with 0.5 mM IPTG (final conc.) when culture reaches O.D.₆₀₀ of 1.0 (~3.5 hr). Grow induced culture for 3.5 hr at 37°C.
8. Pellet 500 ml culture by centrifuging 10 min at 5000 rpm using a Beckman JA-14 rotor. Remove supernatant, and a second 500 ml culture can be added to the same tube and centrifuged as above.
9. Remove supernatant and store at -80°C.

Fusion protein extract – should be done at 4°C or on ice

10. Resuspend pellet (from the equivalent of 1L of culture) in 25 ml of resuspension buffer containing protease inhibitors and 5 mM DTT added fresh. Transfer resuspended cells into 50 ml centrifuge tube.
11. Disrupt cells by sonication. Keep tube on ice at all times. Use the pulse mode at setting 3 and sonicate cells for 3x20 sec.
12. Add Tween20 to a final concentration of 0.3%. Incubate at 4°C under constant shaking for 30-60 min.
13. Centrifuge at 20,000g (15,000rpm using the SS-34 rotor) for 30 min at 4°C.
14. Filter supernatant to eliminate cellular debris that did not pellet.
15. Transfer supernatant to a 50 ml tube.

Protein purification – degas all buffers and GSH matrix before setting up the column

16. Add 10 ml of packed matrix to 20 ml glass column. Place the adaptor at an appropriate distance from the top of the matrix in order to reduce the void volume. Hook the column up to the peristaltic pump in the following order: buffer, pump, followed by the column.
17. Be sure that there are NO air bubbles in the lines or the column.
18. Equilibrate the matrix with 5x bed volume (50 ml of equilibration buffer). Keep the flow rate at 0.5 ml/min. Steps 15-17 should be done in advance to allow the column to properly equilibrate.
19. Stop the pump between buffer transfers and wait briefly before transferring the collection line to a different buffer.
20. Place the collection line into the 50 ml tube containing the crude protein. (A 250 μ l aliquot of the crude sample should be saved for subsequent analysis.) Keep flow rate at 0.5ml/min.
21. Collect the flowthrough and save a sample for subsequent analysis.
22. Wash the column with 10x bed volume (80-100 ml) of wash buffer.
23. Elute fusion protein with 2x bed volume (20 ml) of elution buffer.
24. Collect eluate in a 50 ml tube.
25. Concentrate protein to 0.5 mg/ml using the Amicon 50,000 MWCO spin column.
26. Check protein concentration using the Bradford method.

Equilibration/Resuspension Buffer

50 mM HEPES
3 mM EDTA
50 mM NaCl
10% glycerol
pH 7.5

Add prior to use (final conc.)

10 μ g/ml Pepstatin A (from 1 mg/ml stock in ethanol)
10 μ g/ml Leupeptin (from 1 mg/ml stock in water)
100 μ g/ml PMSF (from 10 mg/ml stock in isopropanol)
5 mM DTT (from 1M stock in water)

Column Wash Buffer

50 mM HEPES
3 mM EDTA
150 mM NaCl
10% glycerol
pH 7.5

Elution Buffer

50 mM HEPES
3 mM EDTA
150 mM NaCl
10% glycerol
10 mM glutathione
pH 8.0

Appendix B4

Standard Operating Procedure for the Rat Estrogen Receptor Equilibrium Exchange Assay

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Rat Estrogen Receptor Equilibrium Exchange Assay

Standard Operating Procedure

1.0 Purpose & Applicability

The purpose of this SOP is to outline a procedure for the quantitation of estrogen receptor number and binding affinity in ovariectomized adult female rat reproductive tissue (i.e., uterus). As tissue receptor number is finite, the binding of ligand to the receptor (i.e., specific binding) is a saturable process. Unsaturable binding of ligand is called nonspecific binding and is due to ligand binding to non-receptor proteins, etc. Total binding is saturable binding + unsaturable binding. Total and nonspecific binding are determined empirically, while specific binding is calculated as their difference (i.e., total - nonspecific). The assay described below measures the binding of radiolabeled synthetic ligand (i.e., [³H]-Estradiol) by cytosolic and/or nuclear receptor extracts. Total [³H]-Estradiol binding is determined by incubating the extracts with increasing concentrations of [³H]-Estradiol during which time the labeled ligand binds to the unoccupied receptors in the cytosol extract or exchanges with endogenous hormone bound to the nuclear receptors. The total bound ligand (i.e., saturable + nonsaturable binding) is separated from free ligand via hydroxylapatite extraction, eluted from the receptor with ethanol and quantified using liquid scintillation counting. Nonspecific binding is determined exactly as above except that a 100-300 fold molar excess of radioinert estradiol is included in each incubation together with the increasing concentrations of [³H]-Estradiol (i.e., binding of [³H]-Estradiol in the presence of a 100-300 fold molar excess of radioinert estradiol represents unsaturable binding). Specific binding is calculated as total - nonspecific binding and is analyzed via Scatchard analysis.

2.0 Safety and Operating Precautions

All procedures with radioisotopes should follow the regulations and procedures as described in the Hazardous Agent Protocol (HAP) and in the Radiation Safety Manual and Protocols.

3.0 Equipment and Materials

3.1 Equipment

- * Corning Stir/Hot Plates
- * Digital Pipets
- * Balance
- * Polytron PT 35/10 Tissue Homogenizer
- * Beckman HPLC with on-line Radiochromatograph
- * Vacuum Concentrator
- * Hamilton Syringes (50 µl)
- * Refrigerated General Laboratory Centrifuge
- * Beckman LX Ultracentrifuge with 90 TI Rotor
- * pH Meter with Tris-compatible electrode
- Scintillation counter

3.0 Equipment and Materials (cont.)

3.2 Chemicals

- * Tris HCL
- * Tris Base
- * Glycerol (99% +)
- * Ethylenediaminetetraacetic Acid (EDTA); Disodium Salt
- * Dithiothreitol (DTT)
- * Hydroxylapatite (Bio-Rad)
- * Scintillation Cocktail
- * Ethyl Alcohol, anhydrous
- * [³H]-Estradiol
- * Radioinert Estradiol (Steraloids; recrystallized)
- * Steroids (Steraloids; recrystallized)

3.3 Supplies

- * 20 ml Polypropylene Scintillation vials
- * 12x75 mm Borosilicate glass test tubes
- * 1000 ml graduated cylinders
- * 500 ml erlenmeyer flasks
- * yellow (0-200 µl) pipet tips

4.0 Methods

4.1 Preparation of TEDG Stock Solutions

(A) 200 mM EDTA

Add 7.444 g EDTA (disodium salt) to 100 ml ddH₂O. Store at 4°C

Use 750 µl/100 ml TEDG buffer = 1.5 mM final concentration of EDTA

(B) 1.0 M Tris

Add in a volumetric Flask:

147.24 g Tris HCL

8.0 g Tris Base

800 ml ddH₂O

Stir until dissolved. QS to 1.0 Liter. Refrigerate to 4°C and then pH the cooled solution to 7.4. pH using standardizing solutions which are also at 4°C. Store at 4°C. Use 1.0 ml of 1.0 M Tris/100 ml TEDG buffer = 10 mM final Tris concentration.

4.2 Preparation of TEDG Buffer (pH 7.4)

Add the following in this order:

<u>Ingredient</u>	<u>To make 100 ml</u>	<u>To make 500 ml</u>	<u>To make 1.0 L</u>	<u>To make 2.0 L</u>
dd H ₂ O	87.15 ml	435.75 ml	871.5 ml	1743.0 ml
1.0 M Tris	1.00 ml	5.00 ml	10.0 ml	20.0 ml
Glycerol	10.00 ml	50.00 ml	100.0 ml	200.0 ml
200 mM EDTA	750 µl	3.75 ml	7.5 ml	15.0 ml

Immediately prior to use in the assay, add:

15.4 mg Dithiothreitol/100 ml TEDG buffer. pH final solution to make sure it is 7.4 at 4 °C

4.3 Preparation of 50 mM Tris Buffer

<u>Ingredient</u>	<u>To make 1.0 L</u>	<u>To make 2.0 L</u>	<u>To make 5.0 L</u>
1.0 M Tris	50 ml	100 ml	250 ml
ddH ₂ O	950 ml	1900 ml	4750 ml

Store at 4 °C. pH final solution to make sure it is 7.4 at 4°C

4.4 Preparation of 60% Hydroxylapatite (HAP) Slurry

Shake Bio-Rad HT-GEL until all the HAP is in suspension (i.e., looks like milk). The evening before the receptor extraction, pour 100 ml of the suspension into a 100 ml graduated cylinder, parafilm seal the top and place in the refrigerator for at least 2 hours. Pour off the phosphate buffer supernatant and bring the volume up to 100 ml with 50 mM Tris buffer. Resuspend the HAP by sealing the top of the graduated cylinder with parafilm and inverting the cylinder several times. Place in the refrigerator overnight. The next morning, repeat the washing steps twice more with fresh 50 mM Tris buffer. After the last wash, add enough 50 mM Tris buffer to make the final solution a 60% slurry (i.e., if the volume of settled HAP is 60 ml, bring the final volume of the slurry up to 100 ml). Store at 4°C until ready for use in the extraction.

4.5 Preparation of [³H]-Estradiol Stock Solutions

Dilute the original 1.0 mCi/ml stock of [³H]-Estradiol to 0.1 µM (i.e., 1×10^{-7} M). This is most easily accomplished by pipeting 1 µl of the stock solution for every specific activity unit (Ci/mmol) and diluting this to 10.0 ml with ethanol. Thus, if the specific activity of the stock vial = 86 Ci/mmol, then pipet 86.0 µl into an amber colored vial and add 10.0 ml ethanol to the vial; this solution is 1×10^{-7} M.

Calculation Check:

$$\begin{aligned}
 86 \mu\text{l} \times 1.0 \text{ mCi}/1000 \mu\text{l} &= 86 \times 10^{-3} \text{ mCi Estradiol} = 86 \times 10^{-6} \text{ Ci Estradiol} \\
 86 \times 10^{-6} \text{ Ci} \div 86.0 \text{ Ci/mmol} &= 1 \times 10^{-6} \text{ mmol Estradiol} = 1 \times 10^{-9} \text{ moles Estradiol} \\
 1 \times 10^{-9} \text{ moles Estradiol} \div .010 \text{ liters} &= 1 \times 10^{-7} \text{ moles/liter} = 0.1 \mu\text{M}
 \end{aligned}$$

To prepare the 1×10^{-8} M stock, simply make a 10-fold dilution of the 1×10^{-7} M stock. To do this, pipet 1.0 ml of the 1×10^{-7} stock into a clean amber colored vial and add 9 ml ethanol. Final concentrations = 0.01 μ M.

4.6 Preparation of 100X Radioinert Estradiol Solutions

Add 27.24 mg Estradiol to a 100 ml volumetric flask, QS to 100 ml with 95% ethanol (1×10^{-3} M or 1 mM stock).

Take 1.0 ml of the 1 mM stock estradiol and place in another 100 ml volumetric flask, QS new flask to 100 ml with 95% ethanol (1×10^{-5} or 10 μ M). This is the 10 μ M radioinert estradiol stock and should be stored in the freezer when not in use (storage in 20 ml aliquots works well).

To make the **1.0 μ M radioinert estradiol stock**: Pipet 2.0 ml of the 10 μ M estradiol stock into a vial and dilute to 20 ml with 95% ethanol (1×10^{-6}).

To make the **0.1 μ M radioinert estradiol stock**: Pipet 2.0 ml of the 1.0 μ M estradiol stock into a vial and dilute to 20 ml with 95% ethanol (1×10^{-7}).

4.7 Standard Curve Construction for Saturation and Scatchard Analysis

The first step is to pipet the radioactive ligand (i.e., [3 H]-Estradiol) with and without a 100-fold excess of radioinert estradiol into each tube so that the final concentrations of [3 H]-Estradiol are 7.0, 3.5, 1.17, 0.7, 0.35, 0.117, 0.035, and 0.0117 nM in a 300 μ l total volume. To accomplish this, label tubes and pipet the following into duplicate 12x75 mm borosilicate glass test tubes:

Tube	Volume [3 H]-E ₂ (μ l)	Final Conc. [3 H]-E ₂ (nM)	Volume Radioinert E ₂ (μ l)	Final Conc. Radioinert E ₂ (nM)	Volume of 50 mM Tris (μ l)	Cytosolic Extract (μ l)
1	21 of 1×10^{-7}	7.00	*****	*****	229	50
2	10.5 of 1×10^{-7}	3.50	*****	*****	239.5	50
3	3.5 of 1×10^{-7}	1.17	*****	*****	246.5	50
4	21 of 1×10^{-8}	0.70	*****	*****	229	50
5	10.5 of 1×10^{-8}	0.35	*****	*****	239.5	50
6	3.5 of 1×10^{-8}	0.117	*****	*****	246.5	50
7	10.5 of 1×10^{-9}	0.035	*****	*****	239.5	50
8	3.5 of 1×10^{-9}	0.0117	*****	*****	246.5	50
9	21 of 1×10^{-7}	7.00	21 of 1×10^{-5}	700	208	50
10	10.5 of 1×10^{-7}	3.50	10.5 of 1×10^{-5}	350	229	50
11	3.5 of 1×10^{-7}	1.17	3.5 of 1×10^{-5}	117	243	50
12	21 of 1×10^{-8}	0.70	21 of 1×10^{-6}	70	208	50
13	10.5 of 1×10^{-8}	0.35	10.5 of 1×10^{-6}	35	229	50
14	3.5 of 1×10^{-8}	0.117	3.5 of 1×10^{-6}	11.7	243	50
15	10.5 of 1×10^{-9}	0.035	10.5 of 1×10^{-7}	3.5	229	50
16	3.5 of 1×10^{-9}	0.0117	3.5 of 1×10^{-7}	1.17	243	50

After all ingredients have been pipeted, gently vortex the incubation tubes, place them in the tube rotator at 4°C and incubate for approximately 20 hours. Set rotator speed at approximately 40%. Proceed to **Day 2** of assay instructions.

Note: tubes #1-8 are Total Binding Tubes and tubes #9-16 are Non-specific Binding Tubes

4.8 Estrogen Receptor Assay Procedure (Keep everything at 4°C!!!)

1. Estrogen Receptor Preparation:

- a) Make TEDG buffer (add the DTT and check pH) and place in ice.
- b) Ovariectomize 10-12 Sprague-Dawley rats at least 10 days prior to receptor preparation.
- c) Sacrifice the rats and remove the uterus from each animal. Trim fat from the uteri.
- d) Weigh each uteri and record the data.
- e) Place all the uteri into a homogenization tube containing TEDG buffer at 4°C.
- f) Decant storage buffer from uteri and add 1.0 ml TEDG buffer per 0.1 gm of tissue.
- g) Homogenize the tissue at 4°C with a Polytron homogenizer using 5-sec bursts. Note: Polytron should be cooled prior to use by placing the probe in TEDG buffer in an ice water bath.
- h) Transfer the homogenate to pre-cooled ultracentrifuge tubes, balance and centrifuge at 105,000 x g (approximately 33,000 rpm with TI-90 Beckman ultracentrifuge rotor) for 60 min. at 4°C.
- i) The supernatant contains the cytosolic estrogen receptors. Decant supernatant and assay directly or freeze (-70°C) until ready for use.

Day 1

Note: If constructing a standard curve for saturation and Scatchard analysis, label tubes and pipet reagents as described in section 4.7. If running a competitive binding assay, start at step 3 and proceed from there.

2. Label **duplicate** 12x75 glass tubes.

- a) *Standard Curve:* label tubes 0, NSB, S1, S2, S3, S4, S5

<u>Standard Label</u>	<u>Initial Conc. (M)</u>	<u>Final Conc. (M)</u>
0	0	0
NSB	3×10^{-6}	1×10^{-7}
S1	3×10^{-7}	1×10^{-8}
S2	3×10^{-8}	1×10^{-9}
S3	1×10^{-8}	3.33×10^{-10}
S4	3×10^{-9}	1×10^{-10}
S5	1×10^{-9}	3.33×10^{-11}

b) *Test Chemicals*: Label tubes 1, 2, 3, 4, The concentrations tested can vary, but a potential standard range of concentrations is outlined below. More than one chemical can be run in an assay if desired.

<u>Sample #</u>	<u>Initial Conc (M)</u>	<u>Final Conc. (M)</u>
1	3×10^{-2}	1×10^{-3}
2	3×10^{-3}	1×10^{-4}
3	3×10^{-4}	1×10^{-5}
4	3×10^{-5}	1×10^{-6}
5	3×10^{-6}	1×10^{-7}
6	3×10^{-7}	1×10^{-8}

3. Pipet 10 μ l of [3 H]-estradiol (initial conc. = 3×10^{-8} M; final conc. = 1×10^{-9} M) into all tubes.
4. Pipet 10 μ l of estradiol standard to appropriate standard tubes. The 0 tube receives 10 μ l of ethanol.
5. Pipet 10 μ l of radioinert test chemical to appropriate sample tubes.
6. Pipet 230 μ l of 50 mM Tris buffer into each tube.
7. Pipet 50 μ l of cytosolic estrogen receptor supernatant to all tubes.
8. Place reaction mixture tubes in rotator at 4°C for 20 hours.
9. Before leaving for the day, prepare the first wash of the HAP slurry as described in Section 4.4.

Day 2

10. Finish washing the HAP as described in Section 4.4. Dilute with 50 mM Tris to yield a 60% slurry and transfer contents to a 100 ml Erlenmeyer flask. Place a stir bar in the flask and place the flask into a beaker of ice water. Stir the HAP slurry by placing the beaker on a stir plate.
11. Label duplicate 12x75 glass tubes with standard & sample numbers and place on ice. These are the HAP tubes.
12. While the slurry is constantly being stirred, pipet 500 μ l of the HAP slurry into the cold, pre-labeled 12x75 tubes.
13. Remove the reaction mixture tubes from the rotator and place them in the ice water bath with the HAP tubes.
14. After mixing the contents of the reaction mixture tubes, pipet 200 μ l of each reaction mixture into the appropriately-labeled, duplicate HAP tubes. *Discard remainder of reaction mixture, unless doing the Saturation & Scatchard Analysis.*
15. Vortex the HAP tubes at 5 minute intervals for a total of 20 minutes. *During this incubation, if you are running the Saturation & Scatchard Analysis, pipet 30 μ l of the remaining reaction mixture into duplicate, appropriately-labeled, scintillation vials (these are called the TotalCount Tubes and will be used to estimate the concentration of total [3 H]-estradiol).*
16. Centrifuge the HAP tubes at 4°C for 3-4 minutes at 600 x g (~1700 rpm).
17. Place the tubes back into the ice water bath and aspirate and discard the supernatant. A vacuum aspiration apparatus is helpful with this step.
18. Add 2.0 ml of 50 mM Tris buffer to each HAP tube. Vortex to resuspend HAP pellet and then centrifuge at 4°C for 3-4 minutes at 1700 rpm.

19. Aspirate and discard the supernatant. Repeat Step 18 twice more.
20. After the third wash, aspirate the supernatant. Add 2.0 ml of cold (4°C) 100% ethanol to each HAP tube. Vortex and place in ice for 15 minutes, vortexing at 5 minute intervals.
21. Centrifuge the HAP tubes at 4°C for 10 minutes at 1700 rpm.
22. Decant the supernatant into appropriately-labeled scintillation vials.
23. Add 10 ml of scintillation cocktail to each vial, cover and shake.
24. Place into scintillation counter and count DPMs.

Evaluation of Data

25. The counts of tube >0' (no radioinert compound added) serve as the comparator for the counts from tubes into which radioinert test compound was added.
26. Determine the percentage of binding: Number of counts from the HAP sample tubes divided by the number of counts from the >0' tube.
27. Plot the percentage of binding as a function of the concentration of the radioinert compound.
28. Determine the IC₅₀ by using the biostatistics program KELL or by noting where the binding curve intersects the 50% value of the ordinate.
29. The relative binding affinity (RBA) of each chemical is determined by:

$$\text{RBA} = \text{IC}_{50} \text{ Estradiol} / \text{IC}_{50} \text{ Test Compound}$$

5.0 Data Processing

5.1 Free Concentration of [³H]-estradiol

Multiply the DPM in the total count tubes by 1.8047×10^{-5} . **NOTE:** This number will change as the specific activity of new batches of isotope change. Use the equation below to calculate the new number for each batch of isotope.

This value will yield the free concentration (i.e., nM) of [³H]-estradiol initially present in each incubation tube.

Calculation Check

$$(\mathbf{X} \text{ DPM} \div 2.22 \times 10^{12} \text{ dpm/Ci}) = (4.5045 \times 10^{-13} \text{ Ci} \div \text{Specific Activity of } [^3\text{H}]\text{-estradiol Ci/mole})$$

$$= (5.4141 \times 10^{-15} \text{ mmole} \div 1000 \text{ mmole/mole}) = (5.4141 \times 10^{-18} \text{ moles} \div \text{Volume of reaction mixture})$$

$$= (1.8047 \times 10^{-14} \text{ moles/liter} \div 1 \times 10^{-9} \text{ moles/nmole}) = \mathbf{X} = 1.8047 \times 10^{-5} \text{ nM}$$

- Notes:**
1. 2.22×10^{12} = a nuclear constant
 2. Specific Activity will vary between batches of [³H]-estradiol
 3. 1000 mmole/mole is used to convert mmoles to moles

4. Volume of reaction mixture should be in Liters
5. 1×10^{-9} moles/nmole is used to convert moles to nmoles

5.2 Calculation of Total, Nonspecific and Specific [^3H]-Estradiol Binding

* Total binding = (Σ DPM from the tubes that contained only [^3H]-estradiol $\times 1.6242 \times 10^{-2}$). This value will be total binding in fmoles.

* Nonspecific binding = (Σ DPM from the tubes containing both [^3H]-estradiol + 100-fold molar excess radioinert estradiol $\times 1.6242 \times 10^{-2}$). This value will be nonspecific binding in fmoles.

* Specific binding = (fmoles total binding - fmoles nonspecific binding)

Calculation Check

To get fmoles, multiply the DPM values by 1.6242×10^{-2} . This is simply nM $\times 300$.

$$[1.0847 \times 10^{-5} \text{ nM} \times (\text{Volume counted} \div 1 \times 10^{-6} \text{ nmoles/fmole})] = 1.6242 \times 10^{-2} \text{ fmoles}$$

Note: The value 1.6242×10^{-2} will change with specific activity of the [^3H]-estradiol batch and the volume of the reaction mixture counted.

5.3 Graphical Presentation of the Data

Maximal binding capacity (B_{max}) and association/dissociation constants (K_a/K_d) can be estimated using a number of commercially available iterative nonlinear regression analysis programs. One of the better programs was developed by Munson and Rodbard and is called LIGAND (Munson PJ, Rodbard D. Anal. Biochem. 1980; 107:220-239).

6.0 References

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Appendix B5

Protocol for the Estrogen Receptor Competitive Binding Assay Using Rat Uterine Cytosol

**(Provided by Dr. Susan Laws, U.S. EPA, NHEERL, Research
Triangle Park, NC, USA and Mr. Gary Timm, U.S. EPA,
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Protocol for the Estrogen Receptor Competitive Binding Assay Using Rat Uterine Cytosol

Purpose of Assay: This assay can be used to determine the relative binding affinities of environmental chemicals for the estrogen receptor (ER α , ER β) as compared to 17 β -estradiol. Data produced using this assay can be used (1) as a screening tool to detect chemicals with possible estrogenic or anti-estrogenic properties; and (2) for development of Quantitative Structure Activity Relationship models to predict the ability of a chemical to bind to the ER.

Distribution of protocol: A final version of this protocol will be distributed as a guide to multiple laboratories, some with previous experience in conducting receptor binding assays, and others with limited or no experience. This protocol is intended to serve as a guide by providing sufficient information to successfully conduct the assay, yet not being totally definitive so that labs already proficient in conducting the assay would be prevented from using well-documented procedures currently in use in their labs.

Terminology:

- E2: estradiol, 17 β -estradiol, inert estradiol
- ^3H -E2: radiolabeled estradiol, [2,3,6,7,16,17- ^3H (N)]-estradiol
- TEDG: Assay buffer (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.4)
- HAP: Hydroxylapatite
- DES: Diethylstilbestrol

I. Preparation of Rat Uterine Cytosol

a. Prepare TEDG buffer (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.4). Dithiothreitol should be added just prior to use.

b. Uterine cytosol should be prepared using uteri from female rats ovariectomized 7 -10 days prior to being killed. Consistency should be maintained for all assays with respect to the age and strain of the females. Fat and mesentary should be quickly trimmed from the uterus. Weigh and record weight of each uterus. Uteri may be used immediately or rapidly frozen on dry ice or in liquid nitrogen, and stored at -80°C for up to 3 months.

c. Prepare uteri for homogenization using ice-cold TEDG buffer at a ratio of 0.1 g of tissue (e.g., use trimmed tissue necropsy weight obtained in step 1b) per 1.0 mL TEDG buffer.

d. Homogenize the tissue using a Polytron homogenizer (5-sec bursts). *Note:* Probe of polytron should be cooled prior to homogenizing each sample by placing the probe in ice-cold TEDG buffer. If possible, the homogenization tube should be kept in an ice-cold water bath during the homogenizing process.

e. Transfer the homogenate to pre-cooled centrifuge tubes and centrifuge for 10 min. at $2,500 \times g$ at 4°C . (The pellet will contain the nuclear fraction and the supernatant will be used for the cytosolic preparation).

f. Transfer the supernatant to pre-cooled ultracentrifuge tubes, balance the tubes and centrifuge at $105,000 \times g$ for 60 min. at 4°C .

g. Combine the supernatant (i.e., cytosol containing ER) and aliquot for immediate use in ER binding assay or for freezing at -80°C . *Note:* cytosol can be frozen for 1 month prior to use in ER binding assay. Do not thaw and re-freeze the cytosol.

h. Determine the protein content for each batch of cytosol using the BioRad Protein Assay Kit (BioRad Chemical Division, Richmond, CA). *Note:* The dithiothreitol in the buffer is not compatible with the Pierce BCA Protein Assay. Typical protein values are 4 -7 mg/mL.

II. Standardization of Methods for ER Binding Assay

Prior to routinely conducting the ER competitive binding assays, the methods should be standardized within each laboratory. This may be accomplished in two steps. First, a series of saturation radioligand binding assays should be conducted to demonstrate ER specificity and saturation. Nonlinear regression analysis of these data and subsequent Scatchard plots will document ER binding affinity (k_d) and number (B_{\max}). Second, a series of ER competitive binding assays should be conducted using chemicals with known affinities for the ER, such as inert E2, DES, estrone. Comparison of IC_{50} s (e.g., the concentration of a chemical that inhibits $^3\text{H-E2}$ binding by 50%) from these assays with reported values in the literature will assist in documenting that the methods are appropriate for routine use in the laboratory.

A. Saturation Radioligand Binding Assay. ER saturation binding experiments measure total, non-specific and specific binding of increasing concentrations of $^3\text{H-E2}$ under conditions of equilibrium. A graph of specific $^3\text{H-E2}$ binding vrs. radioligand concentration should reach a plateau for maximum specific binding indicative of saturation of the ER with the radioligand. In addition, analysis of the data should document the binding of the $^3\text{H-E2}$ to a single, high, affinity binding site (e.g, $K_d = 0.05 - 0.1 \text{ nM}$).

Although several saturation radioligand assays may need to be conducted before an optimal saturation curve, k_d and B_{\max} are achieved, a good starting point is to use enough cytosol to provide 50 - 100 μg protein per assay tube. The concentration for $^3\text{H-E2}$ should range from 0.03 - 3.0 nM in a total assay volume of 0.5 mL. Non-specific binding should be determined by using 100 x the concentration of radiolabeled E2. Analysis of these data should use a non-linear regression analysis such as RADLIG and LIGAND (KELL, BioSoft, Ferguson, MO), with a final display of the data as a Scatchard plot. Rat uterine cytosol prepared using this protocol will typically yield a k_d of 0.05 - 0.1 nM and B_{\max} of 36 -44 fmol ER/100 μg protein (e.g, 0.072 - 0.088 nM ER when 100 μg protein used in total assay volume of 0.5 mL).

An example of a saturation assay worksheet using increasing concentrations of radioligand is shown below. *Note:* For this example, a stock solution of inert E2 should be prepared in ethanol, with all serial dilutions prepared in assay buffer. All concentrations of ^3H -E2 should be prepared in assay buffer.

<i>Typical Estradiol Saturation Assay</i>									
	$^3\text{H-E2}$			<i>Inert E2</i>			<i>Buffer</i>	<i>Cytosol</i>	
<i>Tube #</i>	<i>Initial [] nM</i>	<i>Vol (μL)</i>	<i>Final [] nM</i>	<i>Initial [] nM</i>	<i>Vol (μL)</i>	<i>Final [] nM</i>	<i>Vol (μL)</i>	<i>Vol (μL)</i>	<i>DPM</i>
1	0.3	50	0.03	-			350	100	
2	0.3	50	0.03	-			350	100	
3	0.6	50	0.06	-			350	100	
4	0.6	50	0.06	-			350	100	
5	0.8	50	0.08	-			350	100	
6	0.8	50	0.08	-			350	100	
7	1.0	50	0.1	-			350	100	
8	1.0	50	0.1	-			350	100	
9	3.0	50	0.3	-			350	100	
10	3.0	50	0.3	-			350	100	
11	6.0	50	0.6	-			350	100	
12	6.0	50	0.6	-			350	100	
13	10	50	1	-			350	100	
14	10	50	1	-			350	100	
15	30	50	3	-			350	100	
16	30	50	3	-			350	100	
17	0.3	50	0.03	30	50	3	300	100	
18	0.3	50	0.03	30	50	3	300	100	
19	0.6	50	0.06	60	50	6	300	100	
20	0.6	50	0.06	60	50	6	300	100	
21	0.8	50	0.08	80	50	8	300	100	
22	0.8	50	0.08	80	50	8	300	100	
23	1.0	50	0.1	100	50	10	300	100	
24	1.0	50	0.1	100	50	10	300	100	
25	3.0	50	0.3	300	50	30	300	100	
26	3.0	50	0.3	300	50	30	300	100	
27	6.0	50	0.6	600	50	60	300	100	
28	6.0	50	0.6	600	50	60	300	100	
29	10	50	1	1000	50	100	300	100	
30	10	50	1	1000	50	100	300	100	
31	30	50	3	3000	50	300	300	100	
32	30	50	3	3000	50	300	300	100	
33	0.3	50	0.03	$^3\text{H-E2}$ only, for determining total dpms					
34	0.3	50	0.03	$^3\text{H-E2}$ only, for determining total dpms					
35	0.6	50	0.06	$^3\text{H-E2}$ only, for determining total dpms					
36	0.6	50	0.06	$^3\text{H-E2}$ only, for determining total dpms					
37	0.8	50	0.08	$^3\text{H-E2}$ only, for determining total dpms					

38	0.8	50	0.08	³ H- E2 only, for determining total dpms	
39	1.0	50	0.1	³ H- E2 only, for determining total dpms	
40	1.0	50	0.1	³ H- E2 only, for determining total dpms	
41	3.0	50	0.3	³ H- E2 only, for determining total dpms	
42	3.0	50	0.3	³ H- E2 only, for determining total dpms	
43	6.0	50	0.6	³ H- E2 only, for determining total dpms	
44	6.0	50	0.6	³ H- E2 only, for determining total dpms	
45	10	50	1	³ H- E2 only, for determining total dpms	
46	10	50	1	³ H- E2 only, for determining total dpms	
47	30	50	3	³ H- E2 only, for determining total dpms	
48	30	50	3	³ H- E2 only, for determining total dpms	

B. ER Competitive Binding Assay. An ER competitive binding assay measures the binding of a single concentration of ³H-E2 in the presence of increasing concentrations of a test chemical. The competitive binding curve is plotted as total ³H-E2 binding vrs the concentration (log units) of the competitor. The concentration of the test chemical that inhibits 50% of the maximum ³H-E2 binding is the IC₅₀. Preliminary experiments should evaluate the effect of the ER concentration of the cytosol, assay volume and ³H-E2 concentration on the IC₅₀ calculation using inert E2. A good starting point for the ER competitive binding assay is to use enough cytosol to provide 50 - 100 µg protein per assay tube, with 0.5 -1.0 nM ³H-E2 in a total assay volume of 0.5 mL. Suggested concentrations for test chemicals with a high affinity for the ER are 1 x 10⁻¹¹ to 1 x 10⁻⁷ M; and 1 x 10⁻¹⁰ to 3 x 10⁻⁴ M for chemicals expected to have a lower binding affinity for the ER. Once assay conditions have been optimized, additional ER competitive binding assays should be conducted to compare chemicals with known affinities for the ER, such as DES, estrone, and ethynyl estradiol (positive controls), and the androgen agonist, R1881 (negative control). (See Pages 13 -14 of this protocol for Example Worksheet: ER Competitive Binding Assay). Data for the inert E2 standard curve and each test chemical should be plotted as the percent ³H-E2 bound versus the molar concentration (log) of competitor. Estimates of IC₅₀ should be determined using appropriate nonlinear curve fitting software such as GraphPad Prism (GraphPad Software, Inc., San Diego, CA). (See Pages 10 -12 of this protocol for additional comments on data analysis).

C. Checklist for Standardizing ER Binding Assays within Laboratory

i. Saturation Assays. If conducting the ER assay is new to the laboratory, several publications cited in the reference section of this protocol will be extremely useful when evaluating the data (e.g., Book edited by Hulme et. al., and the manuals from GraphPad Prism and Biosoft KELL). In general, when evaluating data from the ER saturation assays, the following points should be considered.

- As increasing concentrations of ³H-E2 were used, did the specific binding curve reach a plateau? (e.g, Was maximum specific binding reached indicating saturation of ER with ligand?).
- Did the data produce a linear Scatchard plot? (e.g., Non-linear plots generally indicate a problem with the assay such as ligand depletion (concave plot) or incorrect assessment of non-specific binding (convex plot), etc.).

- Is the K_d within an acceptable range (e.g., 0.05 - 0.1 nM)? *Note:* Literature values for K_d using uterine cytosolic preparations have varied from 0.05 - 0.5 nM. The variation in K_d may be a reflection of different labs using radiolabeled estradiol with a wide range of specific activity ($^3\text{H-E2}$ vrs $^{125}\text{I-E2}$). In addition, publications by Salomonsson et al. (1994) and Kuiper et al. (1997, 1998) suggest that a lower K_d may be observed when assays conditions minimize ligand depletion, and that slightly different K_d s exist for ER and ER .
- Are the standard errors for the K_d or B_{\max} too high? Divide the standard error (SE) of the k_d by the k_d , and the SE of the B_{\max} by the B_{\max} . If either ratio is much larger than 20%, then the methods for the assay should be re-evaluated (GraphPad Prism Manual, 1999).
- Is non-specific binding too high? The value for non-specific binding should be less than 50% of the total binding (GraphPad Prism Manual, 1999).

ii. Competitive Binding Assays. Again, if the assay is new to the laboratory, it is suggested that the publications cited in the reference section be utilized to facilitate adequate evaluation of the data. In general, the assay should demonstrate that increasing concentrations of inert E2 can compete with a single concentration of $^3\text{H-ER}$ for binding to the ER. Specific questions to evaluate are as follows:

- As a safeguard against ligand depletion, was the total maximal binding no greater than 10% of the amount of $^3\text{H-E2}$ added per assay tube?
- Was the IC_{50} for inert E2 reasonable? (e.g The IC_{50} for inert E2 should be approximately equal to the molar concentration of $^3\text{H-E2}$ used in the assay tube plus the K_d (determined by nonlinear analysis and Scatchard plot of data obtained from saturation radioligand binding assays).
- Are the data easily replicated with respect to IC_{50} s for inert E2, and selected test chemicals?

III. Estrogen Receptor Competitive Binding Assay: Working Protocol

1.0 Preparation of Assay Buffer

TEDG Buffer (10 mM Tris, 1.5 mM EDTA, 1.0 mM dithiothreitol, 10% glycerol).
Prepare buffer without dithiothreitol, adjust to pH 7.4 and store at 4°C.
Add dithiothreitol just prior to use in assay.

2.0 Preparation of Trace

[2,3,6,7,16,17- $^3\text{H(N)}$]-estradiol ($^3\text{H-E2}$)
New England Nuclear (DuPont) No. NET-517
Store at 4 - 5°C in original container.
Specific Activity (SA) may change with lot.
Obtain the highest specific activity available from the vendor.
Example: Lot # 33639215, Certification Date 5/16/01;
SA = 118 Ci/mmol (261,960 DPM/pmol).
Concentration: 1.0 mCi/mL
Note: SA should be adjusted for decay over time.

Dilute trace with TEDG assay buffer.

Each assay tube should contain 0.5 - 1 nM final concentration of $^3\text{H-E2}$.

3.0 Selection of Receptor Concentration and Assay Volume

a. Receptor concentration of the cytosol and assay volume per assay tube should be adjusted to minimize the likelihood of ligand depletion (e.g., Ligand depletion occurs when a high percentage of the $^3\text{H-E2}$ is bound to ER causing the concentration of the unbound (*free*) $^3\text{H-E2}$ to significantly differ from the concentration of $^3\text{H-E2}$ that was originally added to the assay tube. Hulme and Birdshall, 1992). A general rule of thumb is to optimize the assay conditions so that the ratio of the total $^3\text{H-E2}$ bound in the absence of competitor, to the total $^3\text{H-E2}$ added to each assay tube, is no more than 10%. Decreasing the amount of cytosolic protein and/or increasing the assay volume will generally lower this ratio. Serial dilutions of the cytosol to obtain 50 - 150 μg protein per assay tube in a total assay volume of 500 μL is a good starting point for determining the optimal ER concentration.

4.0 Preparation of E2 for the Standard Curve and non-specific binding (NSB).

a. Standard Curve: A standard curve using inert E2 should be prepared for each ER competitive binding assay. Final concentrations of inert E2 in the assay tubes should range from 1.0×10^{-7} to 1.0×10^{-11} M. Prepare serial dilutions of E2 in ethanol (200 proof) to achieve the Final Concentrations shown below. Use siliconized glass tubes when preparing the standards. The following table shows recommended concentrations for the inert E2 standard curve.

Example of Preparation Procedure for Inert E2 Standard Curve

Concentrations for Inert E2 Standard Curve		
<i>Standards</i>	<i>Initial E2 Concentration (Molar)</i>	<i>*Final E2 Concentration (Molar) in ER assay tube</i>
Negative Control	0 (Inert R1881)	0
0	0 (EtOH)	0
NSB	5×10^{-6}	1×10^{-7}
S1	5×10^{-7}	1×10^{-8}
S2	5×10^{-8}	1×10^{-9}
S3	1.67×10^{-8}	3.33×10^{-10}
S4	5×10^{-9}	1×10^{-10}
S5	1.67×10^{-9}	3.33×10^{-11}
S6	5×10^{-10}	1×10^{-11}
* When 10 μl of each standard is added to the ER assay tube, the final concentration will be as indicated when the total volume in the ER assay tube is 500 μL .		

b. Negative control: It is recommended a chemical be selected as a negative control (e.g., does not demonstrate any binding affinity for the ER), and one concentration of the chemical be tested in each competitive binding assay. R1881, an androgen agonist, at a final concentration of 1×10^{-7} M is used in this protocol as the negative control.

5.0 Preparation of Test Chemicals

a. Stock Solutions: Test chemicals should be diluted in ethanol (200 proof) to 3.0×10^{-2} M (i.e., 30 mM). Use siliconized glass tubes when preparing dilutions. *Note:* Some test chemicals will not be soluble at this concentration, so adjustments will need to be made in the final concentration of the serial dilution tubes depending upon the specific chemical. Likewise, some chemicals may not be soluble in ethanol at all, so appropriate modifications in the ER assay should be made to accommodate any change in solvent.

b. Prepare serial dilutions of each test chemical in ethanol to yield the final concentrations as indicated below. *Note:* The serial dilutions shown in Table 2 are based upon the addition of 10 μ l of each serial dilution of the test chemical in a final assay volume of 500 μ l.

Caution: No more than 0.2% ethanol should be used in the assay tubes.

Table 2 – Test Chemical Concentrations		
<i>Serial Dilutions of Test Chemical</i>	<i>Initial Concentration (Molar)</i>	<i>*Final Concentration in ER assay tube (Molar)</i>
Concentration 1	15×10^{-3}	3.0×10^{-4}
Concentration 2	5.0×10^{-3}	1.0×10^{-4}
Concentration 3	5.0×10^{-4}	1.0×10^{-5}
Concentration 4	5.0×10^{-5}	1.0×10^{-6}
Concentration 5	5.0×10^{-6}	1.0×10^{-7}
Concentration 6	5.0×10^{-7}	1.0×10^{-8}
Concentration 7	5.0×10^{-8}	1.0×10^{-9}
Concentration 8	5.0×10^{-9}	1.0×10^{-10}
<i>*Final Concentration of test chemical in assay tube when 10 μl of Initial Concentration is used in a total volume of 500 μl.</i>		

6.0 Preparation of ER Assay Tubes

- a. Label 12 x 75 mm round bottom assay tubes (siliconized glass) in duplicate as follows:
- 0, NSB, Neg, S1, S2, S3, S4, S5, S6
 - Unknown chemical 1- Concentration 1 (e.g., U1-C1, U1-C2, U1-C8)
 - Unknown chemical 2 -Concentration 1 (e.g., U2-C1, U2-C2,U2-C8)
 - Total DPMS: TC

- b. Place assay tubes in ice bath and add the following to each tube:

Components of ER Competitive Binding Assay		
50	μL	Adjust amount of uterine cytosol to provide 50 - 100 μg protein/assay tube
430	μL	TEDG Assay Buffer
10	μL	³ H-E2 to yield final concentration of 0.5 - 1.0 nM
10	μL	Inert E2, negative control, or test chemical
500	μL	Total volume in each assay tube

c. Vortex assay tubes. (*Note:* Make sure that all components are concentrated at the bottom of tube. If any of the liquid remains on the side of the tube, centrifuge assay tubes for 1 minute at 600 x g (4°C) to concentrate fluid at bottom of tube.)

d. Incubate assay tubes at 4° C for 18 to 20 h. Assay tubes should be placed on a rotator during the incubation period.

6.0 Preparation of 60% Hydroxylapatite (HAP) Slurry

a. The day before beginning this step to separate the bound and free ³H-E2, add 10 g HAP (BioRad) to 100 mL TEDG buffer and gently mix. Cap the container and place the HAP slurry in the refrigerator overnight. (This amount of HAP will generally yield enough slurry for 70 - 100 assay tubes.)

b. The next morning aspirate the supernatant and resuspend the HAP in fresh TEDG buffer. Allow HAP to settle and repeat wash. If HAP is prepared in a graduated cylinder, the amount of buffer needed to prepare a 60% HAP slurry can be estimated using the scale on the outside of the cylinder.

c. After the last wash, resuspend the HAP to a final volume of 60% HAP and 40% buffer. The HAP slurry should be well suspended and ice cold when used in the separation procedure.

7.0 Separation of Bound ³H-E2-ER and Free ³H-E2

Note: To minimize dissociation of bound ³H-E2 from the ER during this process, it is extremely important that the buffers and assay tubes be kept ice-cold and that each step be conducted quickly.

a. Remove ER assay tubes from rotator and place in an ice-water bath. Using an Eppendorf repeating pipet, quickly add 250 μL of HAP slurry (60% in TEDG buffer, well mixed prior to using) to each assay tube.

b. Vortex the tubes at 5 minute intervals for a total of 15 minutes. (*Note:* This is best accomplished by vortexing an entire rack of tubes at once. It is important to keep the assay tubes cold at this point.)

c. Following the incubation period (step 7b), add 2.0 mL of the TEDG assay buffer, quickly vortex, and centrifuge at 4°C for 10 minutes at 1000 x g.

d. At the end of the centrifugation, immediately decant the supernatant (e.g., containing the free ³H-E2. The HAP pellet will contain the bound ³H-E2-estrogen receptors). *Note:* This step can be accomplished quickly by placing the assay tubes in a decanting tube racks. All tubes in the rack can be decanted at once, and the tubes immediately placed back in the ice bath.

e. Add an additional 2.0 mL TEDG ice-cold buffer and vortex briefly to resuspend pellet. Work quickly and keep assay tubes cold. Centrifuge again at 4°C for 15 minutes at 1000 x g.

f. Again quickly decant and discard supernatant. Repeat the wash and centrifugation steps once more.

g. After the final wash, decant the supernatant. Allow the assay tubes to drain briefly for 1 - 5 minutes. (*Note:* Watch carefully in case the HAP pellet begins to run down the side of assay tube which may occur if protein concentration in the cytosol is quite low.) At this point, the separation of the free ³H-E2 and bound ³H-E2-ER has been completed. Assay tubes may be left at room temperature.

8.0 Extraction and Quantifying ³H-E2 bound to ER

a. Add 1.5 ml of ethanol (100%) to each assay tube. Allow the tubes to sit at room temperature for 15 - 20 minutes, vortexing at 5 minute intervals.

b. Centrifuge the assay tubes for 10 minutes at 1000 x g. (Centrifuge can be set at 4°C, but keeping the assay tubes cold is no longer critical at this point.)

c. Pipet an aliquot (usually 1.0 -1.5 ml) or decant the supernatant into 20 mL scintillation vials. Add 10 mL scintillation cocktail, cap and shake vial. *Note:* If a 1.0 ml aliquot is used for scintillation counting, the DPMs should be adjusted to account for the total radioactivity in 1.5 ml (e.g., DPMs x 1.5 = Total DPMs bound).

d. Place vials in scintillation counter for determination of DPMs/vial with quench correction.

9.0 Data Analysis

a. Terminology:

Total $^3\text{H-E2}$: DPMs added to each assay tube

(e.g., can be converted to concentration of total $^3\text{H-E2}$ used in the ER assay)

Total (Maximum) Binding: DPMs in the 0 standard tubes.

Nonspecific Binding: DPMs in the NSB standard (i.e., 100 x excess of inert E2)

Specific Binding: DPMs for each concentration of standard or test chemical minus the mean DPM of the NSB tubes.

b. Data Analysis:

i. IC_{50} calculation: Data for the inert E2 standard curve and each test chemical should be plotted as the percent $^3\text{H-E2}$ bound versus the molar concentration (log) of competitor. Estimates of IC_{50} should be determined using appropriate nonlinear curve fitting software such as GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

ii. Relative Binding Affinity (RBA): The RBA for each competitor (test chemical) should be calculated by dividing the IC_{50} for E2 by the IC_{50} of the competitor and expressing as a percent (e.g., RBA for E2 = 100 %).

c. Checklist for Evaluating ER Competitive Binding Assay Data

i. Inert E2 Standard Curve. The assay should demonstrate that increasing concentrations of inert E2 can displace $^3\text{H-E2}$. The IC_{50} for E2 should be approximately equal to the molar concentration of $^3\text{H-E2}$ plus the K_d (determined by Scatchard analysis). (Prism, GraphPad). The IC_{50} s for the inert E2 standard curve should be easily replicated.

ii. IC_{50} s for test chemicals should be easily replicated.

iii. The ratio of total binding in the absence of competitor to the amount of $^3\text{H-E2}$ added per assay tube should not be greater than 10%.

IV. References

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Example worksheet: Includes a standard curve, a test chemical, and quality control measures

Date and time of assay: _____ Person conducting the assay: _____

Radioligand: ^3H -E2 lot number: _____, specific activity: _____ Ci/mmol, _____ DPM/pmol,

Uterine Cytosol batch number _____, Protein concentration _____ mg/mL,
Amt. Protein used per assay vial: _____ ug,

Date uteri harvested _____ : Date of cytosol preparation _____

Posn and tube label		Competitor	Initial Concentration	Receptor (ul)	Buffer	Tracer	Competitor	HAP	Final
		Concentration	(Molar)						(Molar)
1	0	EtOH		50	430	10	10	250	
2	0	EtOH		50	430	10	10	250	
3	NSB	Inert E2	5E-6	50	430	10	10	250	1E-7
4	NSB	Inert E2	5E-6	50	430	10	10	250	1E-7
5	S1	Inert E2	5E-7	50	430	10	10	250	1E-8
6	S1	Inert E2	5E-7	50	430	10	10	250	1E-8
7	S2	Inert E2	5E-8	50	430	10	10	250	1E-9
8	S2	Inert E2	5E-8	50	430	10	10	250	1E-9
9	S3	Inert E2	1.67E-8	50	430	10	10	250	3.33E-10
10	S3	Inert E2	1.67E-8	50	430	10	10	250	3.33E-10
11	S4	Inert E2	5E-9	50	430	10	10	250	1E-10
12	S4	Inert E2	5E-9	50	430	10	10	250	1E-10
13	S5	Inert E2	1.67E-9	50	430	10	10	250	3.33E-11
14	S5	Inert E2	1.67E-9	50	430	10	10	250	3.33E-11
15	S6.	Inert E2	5E-10	50	430	10	10	250	1E-11
16	S6	Inert E2	5E-10	50	430	10	10	250	1E-11
17	Neg.	Inert R1881	5E-6	50	430	10	10	250	1E-7
18	Neg.	Inert R1881	5E-6	50	430	10	10	250	1E-7
19	u1-c1	Chemical 1	15E-3	50	430	10	10	250	3E-4
20	u1-c1	Chemical 1	15E-3	50	430	10	10	250	3E-4
21	u1-c2	Chemical 1	5E-3	50	430	10	10	250	1E-4
22	u1-c2	Chemical 1	5E-3	50	430	10	10	250	1E-4
23	u1-c3	Chemical 1	5E-4	50	430	10	10	250	1E-5
24	u1-c3	Chemical 1	5E-4	50	430	10	10	250	1E-5
25	u1-c4	Chemical 1	5E-5	50	430	10	10	250	1E-6
26	u1-c4	Chemical 1	5E-5	50	430	10	10	250	1E-6
27	u1-c5	Chemical 1	5E-6	50	430	10	10	250	1E-7
28	u1-c5	Chemical 1	5E-6	50	430	10	10	250	1E-7
29	u1-c6	Chemical 1	5E-7	50	430	10	10	250	1E-8
30	u1-c6	Chemical 1	5E-7	50	430	10	10	250	1E-8
31	u1-c7	Chemical 1	5E-8	50	430	10	10	250	1E-9
32	u1-c7	Chemical 1	5E-8	50	430	10	10	250	1E-9
33	u1-c8	Chemical 1	5E-9	50	430	10	10	250	1E-10
39	u1-c8	Chemical 1	5E-9	50	430	10	10	250	1E-10
40	0	EtOH		50	430	10	10	250	
41	0	EtOH		50	430	10	10	250	
42	NSB	Inert E2	5E-6	50	430	10	10	250	1E-7
43	NSB	Inert E2	5E-6	50	430	10	10	250	1E-7
44	Neg.	Inert R1881	5E-6	50	430	10	10	250	1E-7
45	Neg.	Inert R1881	5E-6	50	430	10	10	250	1E-7
46	Hot	Total Counts		—	—	10	—	—	
47	Hot	Total Counts		—	—	10	—	—	
48	Hot	Total Counts		—	—	10	—	—	
49	Hot	Total Counts		—	—	10	—	—	
50	Hot	Total Counts		—	—	10	—	—	

Appendix C

Chemical and Product Class Information for the Substances Tested in the *In Vitro* ER Binding Assays

Information Sorted by Substance Name

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Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
4,4'-(1,3-Adamantanediyl)diphenol			Phenol	
2-(1-Adamantyl)-4-methylphenol		41031-50-9	Phenol	
4-(1-Adamantyl)phenol		29799-07-3	Phenol	
Alachlor		15972-60-8	Anilide	Pesticide
Aldosterone		52-39-1	Steroid, nonphenolic	Pharmaceutical
Aldrin		309-00-2	Cyclodiene	Pesticide
-trans Allethrin		584-79-2	Pyrethrin; Pyrethroid	Pesticide
<i>p</i> -(7-Alloxy)-11-ethyldibenzo- [<i>b,f</i>]thiepin-10-yl)phenol		85850-86-8	Stilbene; Phenol	
<i>p</i> -(3-(Alloxy)-11-ethyl-6 <i>H</i> - dibenzo[<i>b,f</i>]thiocin-12-yl)phenol hemihydrate		85850-88-0	Triphenylethylene; Phenol	
<i>p</i> -(2-(Alloxy)-6-ethyl-11,12- dihydroxydibenzo[<i>a,e</i>]cyclooctene-5- yl)phenol		85850-87-9	Triphenylethylene; Phenol	
3-(Alloxy)-10-ethyl-11-(4- hydroxyphenyl)dibenz[<i>b,f</i>]thiepin		85850-85-7	Triphenylethylene	
3-(Alloxy)-10-ethyl-11- phenyldibenz[<i>b,f</i>]thiepin		85850-82-4	Triphenylethylene	
3-(Alloxy)-11-ethyl-12-phenyl 6 <i>H</i> - dibenzo[<i>b,f</i>]thiocin		85850-84-6	Triphenylethylene	
3-(Alloxy)-10-ethyl-11- phenyldibenz[<i>b,f</i>]oxepin		83807-07-2	Triphenylethylene	
3-(Alloxy)-11-ethyl-12-phenyl 5,6- dihydroxydibenz[<i>a,e</i>]cyclooctene		85850-83-5	Triphenylethylene	
Amaranth	Acid red 27	915-67-3	Azo compound	Dye
2-Aminoestratriene-3,17 -diol	2-Aminoestradiol	107900-30-1	Steroid, phenolic	
4-Aminoestratriene-3,17 -diol	4-Aminoestradiol	107900-31-2	Steroid, phenolic	
2-Aminoestratrien-17 -ol		17522-06-4	Steroid, nonphenolic	
4-Aminoestratrien-17 -ol		17522-04-2	Steroid, nonphenolic	
4-Aminophenyl ether	4,4'-Oxydianiline	101-80-4	Aniline	
4- <i>tert</i> -Amylphenol	4- <i>tert</i> -Pentylphenol	80-46-6	Alkylphenol	Chemical intermediate
3 -Androstenediol		25126-76-5	Steroid, nonphenolic	
5 -Androstane-3 ,17 -diol	3 -Androstenediol	1852-53-5	Steroid, nonphenolic	Pharmaceutical
5 -Androstane-3 ,17 -diol	3 -Androstenediol	571-20-0	Steroid, nonphenolic	Pharmaceutical
5 -Androstane-3 ,17 -diol		1851-23-6	Steroid, nonphenolic	Pharmaceutical
5 -Androstanedione	5 -Androstane-3,17-dione	1229-12-5	Steroid, nonphenolic	Pharmaceutical
5 -Androstane-3,17-dione	5 -Androstanedione	5982-99-0	Steroid, nonphenolic	
5 -Androstane-3 -ol-17-one		53-41-8	Steroid, nonphenolic	Pharmaceutical
4-Androstenediol	3 ,17 -Dihydroxy-4-androstene; Androst-4-ene-3 ,17 -diol	1156-92-9	Steroid, nonphenolic	Pharmaceutical

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
5-Androstenediol	3,17-Dihydroxy-5-androstene; Androst-5-ene-3,17-diol; 5-Androstene-3,17-diol	521-17-5	Steroid, nonphenolic	Pharmaceutical
4-Androstenedione	4-Androstene-3,17-dione	63-05-8	Steroid, nonphenolic	Precursor of testosterone and other hormones
Anthracene		120-12-7	Polycyclic aromatic hydrocarbon	None found (air pollutant)
Apigenin		520-36-5	Flavone	Natural product
Aroclor 1221		11104-28-2	Polychlorinated biphenyl	Dielectric fluid
Aroclor 1254		11097-69-1	Polychlorinated biphenyl	Dielectric fluid
Atrazine		1912-24-9	Triazine; Aromatic amine	Pesticide
Aurin	Aurine; Corallin	603-45-2	Diphenolalkane	Chemical intermediate
Baicalein		491-67-8	Flavone	Natural product
Benomyl		17804-35-2	Carbamate; Imidazole	Pesticide
Benz[a]anthracene	1,2-Benzanthracene	56-55-3	Polycyclic aromatic hydrocarbon	
Benzeneacetonitrile -[bis(4-hydroxyphenyl) methylene]		66422-14-8	Diphenolalkane; Bisphenol	Chemical intermediate
Benzo[a]carbazole		239-01-0	Polycyclic aromatic hydrocarbon	
Benzo[c]carbazole			Polycyclic aromatic hydrocarbon	
Benzo[b]fluoranthene		205-99-2	Polycyclic aromatic hydrocarbon	
Benzo[k]fluoranthene		207-08-9	Polycyclic aromatic hydrocarbon	
Benzo[a]fluorene	1,2-Benzofluorene; Chrysofluorene	238-84-6	Polycyclic aromatic hydrocarbon	
Benzo[b]fluorene		243-17-4	Polycyclic aromatic hydrocarbon	
Benzo[b]naphtho[2,1-d]thiophene		239-35-0	Polycyclic aromatic hydrocarbon	
Benzo[b]naphtho[2,3-d]thiophene		243-46-9	Polycyclic aromatic hydrocarbon	
Benzo[ghi]perylene	1,12-Benzoperylene	191-24-2	Polycyclic aromatic hydrocarbon	
Benzo[c]phenanthrene		195-19-7	Polycyclic aromatic hydrocarbon	
Benzo[a]pyrene	3,4-Benzopyrene	50-32-8	Polycyclic aromatic hydrocarbon	
Benzo[e]pyrene	1,2-Benzopyrene; 4,5-Benzopyrene	192-97-2	Polycyclic aromatic hydrocarbon	
Benzyl alcohol	Benzenemethanol	100-51-6	Alcohol	
4-Benzyloxyphenol	Benzyl 4-hydroxyphenyl ether; Benzoquin	103-16-2	Phenol	Pharmaceutical
Benzylparaben	Benzyl 4-hydroxybenzoate; Benzyl <i>p</i> -hydroxybenzoate	94-18-8	Paraben	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Biochanin A		491-80-5	Isoflavone	
Bis(<i>m</i> -acetoxyl)-1,1,2-triphenylbut-1-ene		100808-56-8	Stilbene	
Bis(<i>p</i> -acetoxyl)-1,1,2-triphenylbut-1-ene		100808-54-6	Triphenylethylene	
Bisdexoxyestradiol	Estratriene	1217-09-0	Steroid, nonphenolic	
1,1-Bis-(4-hydroxyphenyl) ethane		2081-08-5	Diphenolalkane	Chemical intermediate
4,4-Bis(4-hydroxyphenyl)heptane		7425-79-8	Diphenolalkane	Chemical intermediate
3,4-Bis(3-hydroxyphenyl)hexane		68266-24-0	Diphenolalkane	Chemical intermediate
3,3-Bis(4-hydroxyphenyl)pentane		3600-64-4	Diphenolalkane; Bisphenol	Chemical intermediate
1,1-Bis(4-hydroxyphenyl)propane		1576-13-2	Diphenolalkane; Bisphenol	Chemical intermediate
2,2-Bis(4-hydroxyphenyl)propanol		142648-65-5	Bisphenol	Chemical intermediate
2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1-trichloroethane	2,2-Bis(4-hydroxyphenyl)-1,1,1-trichloroethane; Dihydroxymethoxychlor	2971-36-0	Organochlorine; Bisphenol	Pesticide metabolite
Bisphenol A	4,4'-Isopropylidenediphenol; 4,4'-(1-Methylethylidene)bisphenol	80-05-7	Bisphenol	Chemical intermediate
Bisphenol A bis(chloroformate)	BPACF	2024-88-6	Bisphenol	Chemical intermediate
Bisphenol A diglycidyl ether	BADGE	1675-54-3	Bisphenol	Chemical intermediate
Bisphenol A diglycidyl ether dimethacrylate	BisGMA	1565-94-2	Acrylate	Chemical intermediate
Bisphenol A dimethacrylate		3253-39-2	Acrylate; Bisphenol	Chemical intermediate
Bisphenol A ethoxylate	E-BPA	68140-85-2	Polyether	Chemical intermediate
Bisphenol A ethoxylate diacrylate	BPA-EDA	64401-02-1	Acrylate	Chemical intermediate
Bisphenol A glucuronide			Bisphenol; Glucuronide	
Bisphenol A propoxylate	P-BPA	37353-75-6	Bisphenol	
Bisphenol AF	2,2-Bis(4-hydroxyphenyl)perfluoropropane	1478-61-1	Diphenolalkane; Bisphenol	Chemical intermediate
Bisphenol B	2,2-Bis(4-hydroxyphenyl)butane	77-40-7	Diphenolalkane; Bisphenol	Chemical intermediate
Bisphenol C	2,2-Bis(4-hydroxy-3-methylphenyl)propane	79-97-0	Diphenolalkane; Bisphenol	Chemical intermediate
Bisphenol C 2		14868-03-2	Diphenolalkane; Bisphenol	Chemical intermediate
Bisphenol E	1,1-Bis(4-hydroxyphenyl)ethane; 4,4'-Ethylenediphenol	6052-84-2	Diphenolalkane; Bisphenol	Chemical intermediate
2,2'-Bisphenol F	Bis(2-hydroxyphenyl)methane	2467-02-9	Bisphenol	Chemical intermediate
4,4'-Bisphenol F		620-92-8	Phenol; Bisphenol	Chemical intermediate
Bisphenol S	4,4'-Sulfonyldiphenol	80-09-1	Bisphenol	Chemical intermediate
16 -Bromo-17 -estradiol		54982-79-5	Steroid, phenolic	
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl)phenoxy]-		107144-85-4	Phenyl ether	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]-		107163-56-4	Phenyl ether	
Butolame		150748-23-5	Steroid, phenolic	
Butyl 4-aminobenzoate		94-25-7	Aniline	Pharmaceutical
<i>n</i> -Butylbenzene		104-51-8	Aromatic hydrocarbon; Alkylbenzene	
<i>sec</i> -Butylbenzene	Benzene, (1-methylpropyl)-	135-98-8	Alkylbenzene	Chemical intermediate
Butyl benzyl phthalate	Benzyl butyl phthalate; <i>n</i> -Butyl benzyl phthalate; Butylbenzyl phthalate ester	85-68-7	Phthalate	Plasticizer
Butylparaben	Butyl 4-hydroxybenzoate; Butyl <i>p</i> hydroxybenzoate	94-26-8	Paraben	Food additive; Pharmaceutical additive
2- <i>sec</i> -Butylphenol	<i>o-sec</i> -Butylphenol	89-72-5	Phenol	
2- <i>tert</i> -Butylphenol		88-18-6	Phenol	
3- <i>tert</i> -Butylphenol		585-34-2	Phenol	
4- <i>sec</i> -Butylphenol		99-71-8	Phenol	Pharmaceutical
4- <i>tert</i> -Butylphenol	<i>p-tert</i> - Butylphenol	98-54-4	Phenol	Chemical intermediate (coatings); Lubricant additive; Antioxidant (soap)
Butyl phthalyl <i>n</i> -butyl glycolate		85-70-1	Phthalate	Plasticizer
Caffeine		58-08-2	Purine	Pharmaceutical; Food additive; Natural product
Carbaryl	1-Naphthyl methylcarbamate	63-25-2	Carbamate; Polycyclic aromatic hydrocarbon	Pesticide
Carbofuran		1563-66-2	Carbamate	Pesticide
Castor oil		8001-79-4	Carboxylic acid	Pharmaceutical
(±)-Catechin		7295-85-4	Flavanone	
Chalcone		94-41-7	Chalconoid	Natural product
Chlordane		57-74-9	Organochlorine	Pesticide
-Chlordane		5103-71-9	Organochlorine	Pesticide
Chlormequat chloride		999-81-5	Organochlorine	Plant growth regulator
2-Chloro-4-amino-6-isopropylamino-1,3,5 triazine		6190-65-4	Triazine	Pesticide
2'-Chloro-4,4'-biphenyldiol	2-Chloro-4,4'-biphenyldiol; 4,4'-dihydroxy-2'-chlorobiphenyl	56858-70-9	Organochlorine; Phenol	
2-Chloro-4-biphenylol	2-Chloro-4-hydroxybiphenyl; 4-hydroxy, 2-chloro biphenyl	23719-22-4	Organochlorine; Phenol	
4-Chloro-4'-biphenylol	4-Chloro-4'-hydroxybiphenyl	28034-99-3	Organochlorine; Phenol	
4-Chloro- <i>m</i> -cresol	4-Chloro-3-methylphenol	59-50-7	Organochlorine; Phenol	Preservative (Glue, gum, paint, ink, leather); Pesticide; Pharmaceutical
2-Chloro-4,6-diamino- <i>S</i> -triazine	Chlorodiaminotriazine 2 4 6 s	3397-62-4	Triazine	Pesticide
2-Chloro-4-ethylamino-6-amino-1,3,5-triazine		1007-28-9	Triazine	Pesticide

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2-Chloro-4-ethylamino-6-(1-hydroxyisopropyl)amino-1,3,5-triazine		142179-80-4	Triazine	Pesticide
2-Chloro-4-isopropylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine		142200-36-0	Triazine	Pesticide
11 -Chloromethylestradiol		71794-60-0	Steroid, phenolic	
2-Chloro-4-methylphenol	2-Chloro- <i>p</i> -cresol; <i>p</i> -Cresol, 2-chloro-	6640-27-3	Chlorinated phenol	
4-Chloro-2-methylphenol	4-Chloro- <i>o</i> -cresol	1570-64-5	Organochlorine; Chlorinated phenol	Chemical intermediate
2-Chlorophenol	<i>o</i> - Chlorophenol	95-57-8	Chlorinated phenol	Chemical intermediate
4-Chlorophenol	<i>p</i> -Chlorophenol; <i>p</i> - Chlorophenic acid; 4-Hydroxychlorobenzene	106-48-9	Chlorinated phenol	Chemical intermediate
Chlorotamoxifen		77588-46-6	Triphenylethylene	
Cholesterol		57-88-5	Steroid, nonphenolic	Natural product (animal); Pharmaceutical
Chrysene	Benzo[<i>a</i>]phenanthrene; 1,2-Benzphenanthrene	218-01-9	Polycyclic aromatic hydrocarbon	
Chrysin		480-40-0	Flavone	Natural product
Cineole	1,8-Cineole; 1,8-Epoxy- <i>p</i> -menthane; <i>p</i> -Menthane, 1,8-epoxy Eucalyptol	470-82-6	Terpene	Fragrance
Cinnamic acid	2-Propenoic acid, 3-phenyl-	621-82-9	Carboxylic acid	Fragrance
<i>cis</i> -Clomiphene	Zuclomiphene	15690-55-8	Stilbene	Pharmaceutical
<i>trans</i> -Clomiphene	Clomiphene	911-45-5	Stilbene	Pharmaceutical
Clomiphene citrate		50-41-9	Triphenylethylene	Pharmaceutical
Colchicine		64-86-8	Amide	Pharmaceutical
Corticosterone	17-Deoxycortisol; 11,12-Dihydroxyprogesterone; 11 ,21-Dihydroxyprogesterone; 11-Hydroxycorticoidosterone	50-22-6	Steroid, nonphenolic	Pharmaceutical
Cortisol		50-23-7	Steroid, phenolic	Pharmaceutical
Coumestrol	2,(2,4-Dihydroxyphenyl)-6-hydroxy-3-benzofurancarboxylic acid -lactone	479-13-0	Coumarin; Phenol	Natural product (phytoestrogen)
<i>p</i> -Cumyl phenol		599-64-4	Phenol	Chemical intermediate
Cyclofenil diphenol		5189-40-2	Bisphenol	Pharmaceutical
Cycloprop[14 <i>R</i> ,15]estra-1,3,5(10)-triene-3,17 -diol, 3',15-dihydro-		73860-54-5	Steroid, phenolic	
Cycloprop[14 <i>S</i> ,15]estra-1,3,5(10)-triene-3,17 -diol, 3',15-dihydro-		105455-76-3	Steroid, phenolic	
Cypermethrin		52315-07-8	Organochlorine; Nitrile; Diphenyl ether	Pesticide
Daidzein	4',7-Dihydroxyisoflavone	486-66-8	Isoflavone	Natural product (phytoestrogen)
<i>m,p</i> '-DDD		4329-12-8	Organochlorine	Pesticide
<i>o,p</i> '-DDD	<i>o,p</i> ' -TDE; Mitotane; 2,4'-Dichlorodiphenyldichloroethane	53-19-0	Organochlorine; Diphenylalkane	Pesticide; Pharmaceutical

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
<i>p,p'</i> -DDD	<i>p,p'</i> -TDE; 1,1-Dichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane	72-54-8	Organochlorine; Diphenylalkane	Pesticide
<i>o,p'</i> -DDE	1,1-Dichloro-2-(2-chlorophenyl)2-(4-chlorophenyl)ethylene	3424-82-6	Organochlorine; Diphenylalkane	Pesticide metabolite
<i>p,p'</i> -DDE	1,1-Dichloro-bis-(4-chlorophenyl)ethylene; 4,4'-DDE	72-55-9	Organochlorine; Diphenylalkane	Pesticide metabolite
<i>o,p'</i> -DDT		789-02-6	Organochlorine	Pesticide
(-)- <i>o,p'</i> -DDT		58633-26-4	Organochlorine	Pesticide
(+)- <i>o,p'</i> -DDT		58633-27-5	Organochlorine	Pesticide
<i>p,p'</i> -DDT	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane	50-29-3	Organochlorine; Diphenylalkane	Pesticide
Dehydroepiandrosterone	Dehydroisoandrosterone; Androstenedione; 5-Androstene-3-one	53-43-0	Steroid, nonphenolic	Pharmaceutical
14-Dehydroestradiol-17		58699-19-7	Steroid, phenolic	
9, 11-Dehydroestradiol			Steroid, phenolic	
14-Dehydroestradiol-17 3-methyl ether		35664-58-7	Steroid, nonphenolic	
14-Dehydroestrone		2119-18-8	Steroid, phenolic	
14-Dehydroestrone 3-methyl ether		17550-11-7	Steroid, nonphenolic	
3-Deoxyestradiol	Estratriene-17 -ol	2529-64-8	Steroid, nonphenolic	
3-Deoxyestrone		53-45-2	Steroid, nonphenolic	Pharmaceutical
(<i>R</i>)-4'-Deoxyindenestrol A		138515-00-1	Stilbene	
(<i>rac</i>)-4'-Deoxyindenestrol A			Stilbene	
(<i>S</i>)-4'-Deoxyindenestrol A		138514-99-5	Stilbene	
(<i>R</i>)-5-Deoxyindenestrol A		138515-02-3	Stilbene	
(<i>rac</i>)-5-Deoxyindenestrol A		138472-84-1	Stilbene	
(<i>S</i>)-5-Deoxyindenestrol A		138515-01-2	Stilbene	
17-Desoxyestradiol	Estra-1,3,5(10)-triene-3-ol; Estratriene-3-ol; 17-Deoxyestrone	53-63-4	Steroid, phenolic	
Dexamethasone		50-02-2	Steroid, nonphenolic	Pharmaceutical
1,3-Diacetoxy-17 -ethynyl-7 -methyl-1,3,5(10)-estratriene-17 -ol			Steroid, nonphenolic	
4,4'-Diaminostilbene dihydrochloride		66635-40-3	Stilbene	
Dibenz[<i>a,h</i>]anthracene		53-70-3	Polycyclic aromatic hydrocarbon	
Dibenzo-18-crown-6	Crown 18; Dibenzocrown; Dibenzo-18-crown-6; Dibenzo-18-crown-6-ether; Dicyclohexano-18-crown-6	14187-32-7	Crown ether	Chemical intermediate
1,3-Dibenzoyloxy-17 -ethynyl-7 -methyl-1,3,5(10)-estratriene-17 -ol			Steroid, nonphenolic	
1,3-Dibenzyltetramethyldisiloxane			Siloxane	
Dibutyl benzyl phthalate			Phthalate	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2,6-Di- <i>tert</i> -butylphenol	4,4'-Methylenebis; 2,6-Bis(1,1-dimethyl)phenol	128-39-2	Alkylphenol	Chemical intermediate
Dibutyl phthalate	Di- <i>n</i> -butyl phthalate ester; Di- <i>n</i> -butyl phthalate; Dibutyl 1,2-benzenedicarboxylate	84-74-2	Phthalate	Plasticizer
2,4'-Dichlorobiphenyl	PCB 8	34883-43-7	Polychlorinated biphenyl	Dielectric fluid
2,5-Dichlorobiphenyl	PCB 9	34883-39-1	Polychlorinated biphenyl	Dielectric fluid
3,4-Dichlorobiphenyl	PCB 12	2974-92-7	Polychlorinated biphenyl	Dielectric fluid
3,5-Dichlorobiphenyl	PCB 14	34883-41-5	Polychlorinated biphenyl	Dielectric fluid
4,4'-Dichlorobiphenyl	PCB 15	2050-68-2	Polychlorinated biphenyl	Dielectric fluid
2,5-Dichloro-2'-biphenylol		53905-30-9	Polychlorinated biphenyl	
2,5-Dichloro-3'-biphenylol		53905-29-6	Polychlorinated biphenyl	
2',5'-Dichloro-4-biphenylol	2',5'-Dichloro-4-hydroxybiphenyl	53905-28-5	Polychlorinated biphenyl	
2,6-Dichloro-4'-biphenylol	4-Hydroxyl, 2',6'-dichloro biphenyl	79881-33-7	Polychlorinated biphenyl	
3,4-Dichloro-2'-biphenylol		209613-97-8	Polychlorinated biphenyl	
3,4-Dichloro-3'-biphenylol		14962-34-6	Polychlorinated biphenyl	
3,4-Dichloro-4'-biphenylol		53890-77-0	Polychlorinated biphenyl	
3,5-Dichloro-2'-biphenylol			Polychlorinated biphenyl	
3,5-Dichloro-4'-biphenylol			Polychlorinated biphenyl	
3,5-Dichloro 2-hydroxy-2-methylbut-3-enalide	M2	16776-82-1	Organochlorine	Pesticide
2,4-Dichlorophenoxyacetic acid	2,4-D	94-75-7	Phenoxy carboxylic acid	Pesticide
2-[[[(3,5-Dichlorophenyl)amino]-carbamoyl]oxy]-2-methyl-3-butenic acid	M1	119209-27-7	Organochlorine	Pesticide
Dieldrin		60-57-1	Organochlorine; Chlorinated cyclodiene	Pesticide
Dienestrol	<i>trans,trans</i> -Dienestrol	84-17-3	Diphenylalkene	Pharmaceutical
-Dienestrol		13029-44-2	Diphenylalkene	
-Dienestrol		35495-11-5	Diphenylalkene	Pharmaceutical
1,3-Diethyl-6,4'-dihydroxy-2-phenylindene			Stilbene	
Di-2-ethylhexyl adipate	Bis(2-ethylhexyl) adipate	103-23-1	Ester	Plasticizer
Diethylhexyl phthalate	Bis(2-ethylhexyl) phthalate	117-81-7	Phthalate	Chemical intermediate
1,3-Diethyl-4-hydroxy-2-phenylindene			Indene	
1,3-Diethyl-6-hydroxy 2-phenylindene			Indene	
<i>meso-p</i> -(, -Diethyl- <i>p</i> -methylphenethyl)phenol		267408-76-4	Phenol	
Diethyl phthalate	1,2-Benzenedicarboxylic acid, diethyl ester; Diethyl 1,2-benzenedicarboxylate	84-66-2	Phthalate	Solvent; Plasticizer; Pesticide

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Diethylstilbestrol		56-53-1	Stilbene	Pharmaceutical
3,3'-Diethylstilbestrol		5959-71-7	Stilbene	
Diethylstilbestrol dimethyl ether	Dimestrol	130-79-0	Stilbene	Pharmaceutical
Diethylstilbestrol epoxide		6052-82-0	Stilbene	
Diethylstilbestrol phenanthrene			Stilbene	
(<i>rac</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon; Phenol	
5,11- <i>trans</i> -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon; Phenol	
(5 <i>R</i> ,11 <i>R</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon; Phenol	
(5 <i>S</i> ,11 <i>S</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon; Phenol	
Dihexyl phthalate		84-75-3	Phthalate	Plasticizer
5,6-Dihydro-8-[2-(dimethylamino)ethoxy]-12-ethyl-11-phenyl-dibenzo[<i>a,e</i>]cyclooctene, hydrate (1:4)		85850-78-8	Triphenylethylene	
Dihydrogenistein	4',5,7-Trihydroxyisoflavan-4-one	21554-71-2	Isoflavone	
Dihydroglycitein	4 <i>H</i> -1-Benzopyran-4-one, 2,3-dihydro-7-hydroxy-3-(4-hydroxyphenyl)-6-methoxy- (9CI)	94105-88-1	Isoflavone	
5 -Dihydrotestosterone	Androstanolone; Stanolone; 4-Dihydrotestosterone; 4,5 -Dihydrotestosterone	521-18-6	Steroid, nonphenolic	Pharmaceutical
5 -Dihydrotestosterone	17 -Hydroxy-5 -androstane-3-one; Etiocholan-17 -ol-3-one	571-22-2	Steroid, nonphenolic	Pharmaceutical
2,2'-Dihydroxybenzophenone		835-11-0	Benzophenone	
2,4-Dihydroxybenzophenone	Benzoresorcinol	131-56-6	Benzophenone	Chemical additive
4,4'-Dihydroxybenzophenone	Bishydroxy-4-phenylketone	611-99-4	Benzophenone; Phenol	Pharmaceutical; Chemical intermediate (monomer)
4,4'-Dihydroxybiphenyl	4,4'-Biphenol	92-88-6	Biphenyldiol	Chemical intermediate
Dihydroxydiethylstilbestrol		7507-01-9	Stilbene	
6,4'-Dihydroxyflavone		63046-09-3	Flavone	
3,3'-Dihydroxyhexestrol		79199-51-2	Diphenolalkane	
2,2'-Dihydroxy-4-methoxybenzophenone	Dioxybenzone	131-53-3	Benzophenone	Chemical additive
3-(2,3 Dihydroxypropoxy)-10-ethyl-11-phenyldibenz[<i>b,f</i>]oxepin		85850-89-1	Triphenylethylene	
Diisobutyl phthalate	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester; Phthalic acid, diisobutyl ester	84-69-5	Phthalate	Plasticizer
Diisodecyl phthalate		26761-40-0	Phthalate	Plasticizer
Diisoheptyl phthalate		41451-28-9	Phthalate	Plasticizer
Diisononyl phthalate		28553-12-0	Phthalate	Plasticizer

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
11-[2-(<i>N,N</i> -Dimethylamino)ethoxy]estra-1,3,5(10)-triene-3,17-diol			Steroid, phenolic	
3-[2-(Dimethylamino)ethoxy]-11-ethyl-12-phenyl)-6 <i>H</i> -dibenzol[<i>b,f</i>]thiactin		85850-79-9	Triphenylethylene	
3-[2-(Dimethylamino)ethoxy]-10-ethyl-11-phenyldibenz[<i>b,f</i>]oxepin		85850-76-6	Triphenylethylene	
7-[2-(Dimethylamino)ethoxy]-11-ethyl-10-phenyldibenz[<i>b,f</i>]thiepin		85850-77-7	Triphenylethylene	
11-[3-(<i>N,N</i> -Dimethylamino)-propoxy]estra-1,3,5(10)-triene-3,17-diol		130043-38-8	Steroid, phenolic	
, -Dimethyl- -ethylallenolic acid		15372-37-9	Polycyclic aromatic hydrocarbon	
2,6-Dimethylhexestrol		334707-28-7	Diphenolalkane	
1,6-Dimethylnaphthalene		575-43-9	Polycyclic aromatic hydrocarbon	
Dimethyl phthalate		131-11-3	Phthalate	Plasticizer; Solvent
, -Dimethylstilbestrol	Dimethylstilbesterol; Dimestrol	552-80-7	Stilbene	Pharmaceutical
Dimethyl sulfoxide	DMSO	67-68-5	Sulfoxide	Solvent
5,11- <i>trans</i> -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5 <i>R</i> ,11 <i>R</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5 <i>S</i> ,11 <i>S</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
Di- <i>n</i> -octyl phthalate		117-84-0	Phthalate	Plasticizer
Diphenolic acid		126-00-1	Diphenolalkane	Chemical intermediate
<i>trans,trans</i> -1,4-Diphenyl-1,3-butadiene	Benzene, 1,1'-(1,3-butadiene-1,4-diyl)bis-	886-65-7	Diphenylalkene	
4-[1,2-(Diphenyl-1-butenyl)]phenol acetate		100808-55-7	Triphenylethylene	
2,3-Diphenylindenone-1			Indene	
4-[1-(Diphenylmethylene)propyl]phenol acetate		82333-68-4	Triphenylethylene	
1,3-Diphenyltetramethyldisiloxane		56-33-7	Siloxane	
5,11- <i>trans</i> -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5 <i>R</i> ,11 <i>R</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5 <i>S</i> ,11 <i>S</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
4-Dodecylphenol	4-Laurylphenol	104-43-8	Alkylphenol	Solvent
Doisynoesrol	2-Phenanthrenecarboxylic acid, 1-ethyl-1,2,3,4-tetrahydro-7-methoxy-2-methyl-, <i>cis</i> -(±)- (9CI)	15372-34-6	Tetrahydrophenanthrene	
Dopamine	4-(Aminoethyl)catechol	51-61-6	Alkylphenol	Pharmaceutical

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Droloxifene	3-Hydroxytamoxifen	82413-20-5	Stilbene; Triphenylethylene	Pharmaceutical
Empenthrin		54406-48-3	Pyrethrin; Pyrethroid	Pesticide
- Endosulfan		959-98-8	Organochlorine	Pesticide
, - Endosulfan	Endosulfan	115-29-7	Organochlorine; Chlorinated cyclodiene	Pesticide
-Endosulfan	Endosulfan 2	33213-65-9	Organochlorine; Chlorinated cyclodiene	Pesticide
16-Epiestriol	1,3,5(10)-Estratrien-3,16 ,17 - triol	547-81-9	Steroid, phenolic	Pharmaceutical
17-Epiestriol	1,3,5(10)-Estratrien-3,16 ,17 - triol	1228-72-4	Steroid, phenolic	
Epitestosterone	Androst-4-en-3-one, 17- -hydroxy-	481-30-1	Steroid, nonphenolic	
Equilenin	<i>d</i> -Equilenin	517-09-9	Steroid, phenolic	Pharmaceutical
Equilin		474-86-2	Steroid, phenolic	Pharmaceutical
Equol		531-95-3	Isoflavone	Pharmaceutical
<i>Erythro</i> -MEA	Erythro- -Ethyl- '-methyl-4,4'- dihydroxybibenzyl	20576-52-7	Bisphenol	
16 -Estradiol	Estratriene-3,16 -diol	1090-04-6	Steroid, phenolic	
17 -Estradiol	Estra-1,3,5(10)-triene-3,17 -diol; Estra-1,3,5(10)-triene-3,17-diol, (17-)-	57-91-0	Steroid, phenolic	
17 -Estradiol	E2; Estradiol; Estratriene-3,17 - diol; Estra-1,3,5(10)-triene-3,17- diol, (17-)-	50-28-2	Steroid, phenolic	Pharmaceutical
9 -Estradiol			Steroid, phenolic	
Estradiol 17-acetate			Steroid, phenolic	
17 -Estradiol 3-acetate		4245-41-4	Steroid, nonphenolic	
Estradiol benzoate		50-50-0	Steroid, nonphenolic	Pharmaceutical
Estradiol diacetate		3434-88-6	Steroid, nonphenolic	Pharmaceutical
17 -Estradiol 3-methyl ether		1035-77-4	Steroid, nonphenolic	Pharmaceutical
9-Estratetraene-3,17 -diol	Estra-1,3,5(10),9(11)-tetraene- 3,17 -diol	791-69-5	Steroid, phenolic	
Estra-1,3,5(10),6-tetraen-17-one, 3- hydroxy-			Steroid, phenolic	
Estra-1,3,5(10)-triene-3,17 - diol,14,15 - epoxy-		79581-12-7	Steroid, phenolic	
Estra-1,3,5(10)-triene-3,17 - diol,14 ,15 -epoxy-		79645-49-1	Steroid, phenolic	
Estra-1,3,5(10)-triene-3,14,17 -triol		16288-09-8	Steroid, phenolic	
Estratriene-3,6 ,17 -triol		1229-24-9	Steroid, phenolic	Pharmaceutical
Estriol	Estratriene-3,16 ,17 -triol; Estratriol; E3	50-27-1	Steroid, phenolic	Pharmaceutical
Estrone	Estratriene-3-ol-17-one; E1	53-16-7	Steroid, phenolic	Pharmaceutical
Estrone 3-acetate		901-93-9	Steroid, nonphenolic	Chemical intermediate

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Estrone 3-methyl ether		1624-62-0	Steroid, nonphenolic	Chemical intermediate
Estrone-3-sulfate		481-97-0	Steroid, nonphenolic	Chemical intermediate
17 -Ethinyl estradiol	Ethinylestradiol; Ethynylestradiol	57-63-6	Steroid, phenolic	Pharmaceutical
17 -Ethinyl estradiol		4717-38-8	Steroid, phenolic	Pharmaceutical
Ethyl cinnamate		103-36-6	Phenylalkene	Fragrance
3-Ethyl-6,4'-dihydroxy-2-phenylindene			Indene	
2-Ethylhexyl paraben	2-Ethylhexyl 4-hydroxybenzoate	5153-25-3	Paraben	Food additive
4-Ethyl-7-hydroxy-3-(methoxyphenyl)-2H -1-benzopyran-2-one		5219-17-0		
3-[(10-Ethyl-11- <i>p</i> -hydroxyphenyl)dibenzo[<i>b,f</i>]oxepin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)		85850-93-7	Triphenylethylene	
3-[(10-Ethyl-11- <i>p</i> -hydroxyphenyl)dibenzo[<i>b,f</i>]thiepin-3-yl)oxy]-1,2-propanediol		85850-94-8	Triphenylethylene	
3-[(11-Ethyl-12-(<i>p</i> -hydroxyphenyl)-6- <i>H</i> -dibenzo[<i>b,f</i>]thiocin-3-yl)oxy]-, hydrate 1,2-propanediol		85864-54-6	Triphenylethylene	
3-[(6-Ethyl-5-(<i>p</i> -hydroxyphenyl)-11,12-dihydrodibenzo[<i>a,e</i>]cycloocten-2-yl)oxy]-1,2-propanediol		85850-95-9	Triphenylethylene	
3-Ethyl-4'-hydroxy-2-phenylindene			Indene	
3-Ethyl-6-hydroxy 2-phenylindene			Indene	
3-Ethyl-4'-hydroxy 2-phenylindenone-1			Indene	
3-Ethyl-6-hydroxy 2-phenylindenone-1			Indene	
3-Ethyl-4-(<i>p</i> -methoxyphenyl)-2-methyl-3-cyclohexene-1-carboxylic acid		1755-52-8	Carboxylic acid	
Ethyl paraben	Ethyl 4-hydroxybenzoate; Ethyl- <i>p</i> -hydroxybenzoate;	120-47-8	Paraben	Preservative (pharmaceuticals); Food additive
2-Ethylphenol	Phenol, <i>o</i> -ethyl-; Phlorol	90-00-6	Alkylphenol	
3-Ethylphenol	Phenol, <i>m</i> -ethyl-	620-17-7	Alkylphenol	
4-Ethylphenol	Phenol, <i>p</i> -ethyl-	123-07-9	Phenol; Alkylphenol	Flavor
3-[(10-Ethyl-11-phenyl)dibenzo- <i>[b,f]</i> thiepin-3-yl)oxy]-1,2-propanediol, complexed with isononyl alcohol 2:1		85850-90-4		
3-[(11-Ethyl-12-phenyl-6 <i>H</i> -dibenzo[<i>b,f</i>]thiocin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)		85850-92-6		
3-[(6-Ethyl-5-phenyl-11,12-dihydrodibenzo[<i>a,e</i>]cycloocten-2-yl)oxy]-1,2-propanediol		85850-91-5		
Eugenol	Phenol, 4-allyl-2-methoxy-	97-53-0	Alkoxyphenol	Pharmaceutical; Cosmetic (fragrance); Chemical intermediate
Fenvalerate	Pydrin; (+)-alpha-Cyano-3-phenoxybenzyl-(+)-alpha-(4-chlorophenyl)isovalerate	51630-58-1	Organochlorine; Diphenyl ether	Pesticide

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Fisetin		528-48-3	Flavone	Natural product
Flavanone		17002-31-2	Flavanone	Natural product
Flavone		525-82-6	Flavone	Natural product
Fluoranthene		206-44-0	Polycyclic aromatic hydrocarbon	
Fluorene		86-73-7	Polycyclic aromatic hydrocarbon	Dye
2-Fluoroestratrien-17 -ol		101772-22-9	Steroid, nonphenolic	
4-Fluoroestratrien-17 -ol		96607-54-4	Steroid, nonphenolic	
2-(2-Fluorophenyl)-3-phenyl-6-hydroxyindene			Indene	
Fluorotamoxifen		73617-96-6	Triphenylethylene	
Folic acid		59-30-3	Pteridine	Pharmaceutical
Formononetin	7-Hydroxy-4'-methoxyisoflavone	485-72-3	Isoflavone	Pharmaceutical; Natural product
Furfural	2-Furaldehyde; 2-Furancarboxaldehyde	98-01-1	Heterocyclic aromatic aldehyde	Pesticide
Genistein	4',5,7-Trihydroxyisoflavanone	446-72-0	Isoflavone	Pharmaceutical; Natural product
Genistin	Genistein glucoside	529-59-9	Isoflavone	Natural product
Glyceollin		66241-09-6	Isoflavone	Natural product
Glycitein		40957-83-3	Isoflavone	
Glycitin			Isoflavone	
Heptachlor		76-44-8	Organochlorine; Chlorinated cyclodiene	Pesticide
2,2',3,3',4',5,6-Heptachlorobiphenyl	PCB 177	52663-70-4	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',4,5,6-Heptachlorobiphenyl	PCB 173	68194-16-1	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',5,5',6-Heptachlorobiphenyl	PCB 178	52663-64-6	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,4',5',6-Heptachlorobiphenyl	PCB 183	52663-69-1	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,4',6,6'-Heptachlorobiphenyl	PCB 184	74472-48-3	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4',5,5',6-Heptachlorobiphenyl	PCB 187	52663-68-0	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4',5,6,6'-Heptachlorobiphenyl	PCB 188	74487-85-7	Polychlorinated biphenyl	Dielectric fluid
2,3,3',4,4',5,6-Heptachlorobiphenyl	PCB 190	41411-64-7	Polychlorinated biphenyl	Dielectric fluid
2,3,3',4',5,5',6-Heptachlorobiphenyl	PCB 193	69782-91-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',4',5,5'-Heptachloro-4-biphenylol		158076-64-3	Polychlorinated biphenyl	
2,2',3,3',4,4',5,5'-Heptachloro-3-biphenylol		158076-69-8	Polychlorinated biphenyl	
2,2',3,4',5,5',6-Heptachloro-4-biphenylol		158076-68-7	Polychlorinated biphenyl	
Heptanal	<i>n</i> -Heptaldehyde	111-71-7	Aldehyde	Fragrance
4-(Heptyloxy)phenol	4-Heptyloxyphenol	13037-86-0	Alkoxyphenol	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Heptyl 4-paraben	Benzoic acid, 4-hydroxy-, heptyl ester; Heptyl <i>p</i> -hydroxybenzoate	1085-12-7	Paraben	Preservative
Hesperetin		520-33-2	Flavanone	Natural product
Hexachlorobenzene		118-74-1	Organochlorine	Chemical intermediate; Pesticide; Plasticizer
2,2',3,3',4,4'-Hexachlorobiphenyl	PCB 128	38380-07-3	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,4',5'-Hexachlorobiphenyl	PCB 138	35065-28-2	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,5,6'-Hexachlorobiphenyl	PCB 143	68194-15-0	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4',5,6'-Hexachlorobiphenyl	PCB 149	38380-04-0	Polychlorinated biphenyl	Dielectric fluid
2,2',3,5,5',6'-Hexachlorobiphenyl	PCB 151	52663-63-5	Polychlorinated biphenyl	Dielectric fluid
2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153	35065-27-1	Polychlorinated biphenyl	Dielectric fluid
2,2',4,4',6,6'-Hexachlorobiphenyl	PCB 155	33979-03-2	Polychlorinated biphenyl	Dielectric fluid
2,3,3',4,4',6'-Hexachlorobiphenyl	PCB 158	74472-42-7	Polychlorinated biphenyl	Dielectric fluid
2,3',4,4',5',6'-Hexachlorobiphenyl	PCB 168	59291-65-5	Polychlorinated biphenyl	Dielectric fluid
3,3',4,4',5,5'-Hexachlorobiphenyl	PCB 169	32774-16-6	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',4',5'-Hexachloro-4-biphenylol		158076-62-1	Polychlorinated biphenyl	
2,2',3,4',5,5'-Hexachloro-4-biphenylol		145413-90-7	Polychlorinated biphenyl	
2',3,3',4',5,5'-Hexachloro-4-biphenylol		158076-63-2	Polychlorinated biphenyl	
<i>n</i> -Hexanol	Hexyl alcohol; 1-Hexanol	111-27-3	Alcohol	Chemical intermediate
Hexestrol	<i>meso</i> -Hexestrol	84-16-2	Diphenolalkane; Bisphenol	Pharmaceutical
<i>DL</i> -Hexestrol		5776-72-7	Diphenolalkane	Pharmaceutical
Hexestrol monomethyl ether		13026-26-1	Alkoxyphenol	
3-Hydroxybenzo[<i>b</i>]naphtho[2,1- <i>d</i>]thiophene			Thiophene	
2-Hydroxybenzo[<i>c</i>]phenanthrene		22717-94-8	Polycyclic aromatic hydrocarbon	
3-Hydroxybenzo[<i>b</i>]phenanthro[2,3- <i>d</i>]thiophene			Thiophene	
4-Hydroxychalcone		20426-12-4	Chalconoid	
4'-Hydroxychalcone		2657-25-2	Chalconoid	
4'-Hydroxychalcone (<i>cis</i> - and <i>trans</i> -)		38239-52-0	Chalconoid	
2-Hydroxychrysene	2-Chrysenol	65945-06-4	Polycyclic aromatic hydrocarbon	
4'-Hydroxy-2,3-diphenylindenone-1			Triphenylethylene	
6'-Hydroxy-2,3-diphenylindenone-1			Triphenylethylene	
11 -Hydroxyestradiol		1464-61-5	Steroid, phenolic	
11 -Hydroxyestradiol		5444-22-4	Steroid, phenolic	
14 -Hydroxyestradiol		60183-66-6	Steroid, phenolic	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2-Hydroxyestradiol		362-05-0	Steroid, phenolic	
4-Hydroxyestradiol		5976-61-4	Steroid, phenolic	
2-Hydroxyestratrien-17 -ol	Estratriene-2,17 -diol	2259-89-4	Steroid, phenolic	
4-Hydroxyestratrien-17 -ol	Estratriene-4,17 -diol	17592-89-1	Steroid, phenolic	
3-Hydroxyestra-1,3,5(10)-trien-16-one		3601-97-6	Steroid, phenolic	
2-Hydroxyestrone		362-06-1	Steroid, phenolic	Pharmaceutical
2-Hydroxyethyl methacrylate		868-77-9	Acrylate	Chemical intermediate
3'-Hydroxyflavanone		92496-65-6	Flavanone	
4'-Hydroxyflavanone		135413-27-3	Flavanone	
6-Hydroxyflavanone		4250-77-5	Flavanone	
7-Hydroxyflavanone		6515-36-2	Flavanone	
6-Hydroxyflavone		6665-83-4	Flavone	
7-Hydroxyflavone		6665-86-7	Flavone	
Hydroxyflutamide		52806-53-8	Nitrobenzene	Pharmaceutical
2-Hydroxy-4-methoxybenzophenone	Oxybenzone	131-57-7	Benzophenone	Pharmaceutical
6-Hydroxy-2'-methoxyflavone		61546-59-6	Flavone	
2-Hydroxy-5-methylchrysene			Polycyclic aromatic hydrocarbon	
8-Hydroxy-5-methylchrysene			Polycyclic aromatic hydrocarbon	
16 -Hydroxy-16-methyl-17 -estradiol 3-methyl ether	16-Methylestra-1,3,5(10)-triene-3,16 ,17 -triol 3-methyl ether	3434-79-5	Steroid, nonphenolic	
4-Hydroxytamoxifen	Hydroxytamoxifen	68047-06-3	Triphenylethylene	Pharmaceutical
6-Hydroxytetralin	5,6,7,8-Tetrahydro-2-naphthol	1125-78-6	Polycyclic aromatic hydrocarbon; Phenol	
ICI 164384		98007-99-9	Steroid, phenolic	Pharmaceutical
ICI 182780		129453-61-8	Steroid, phenolic	Pharmaceutical
Imiprothrin		72963-72-5	Pyrethrin; Pyrethroid	Pesticide
Indanestrol		71855-45-3	Indane	
Indanyldiethylstilbestrol			Stilbene	
Indenestrol A		24643-97-8	Stilbene	
(<i>R</i>)-Indenestrol A		115217-03-3	Stilbene	
(<i>rac</i>)-Indenestrol A		115217-02-2	Stilbene	
(<i>S</i>)-Indenestrol A		115217-04-4	Stilbene	
Indenestrol B		38028-27-2	Stilbene	
(<i>R</i>)-Indenestrol B			Stilbene	
(<i>rac</i>)-Indenestrol B		133830-97-4	Stilbene	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
(<i>S</i>)-Indenestrol B		115217-06-6	Stilbene	
Indeno[1,2,3- <i>cd</i>]pyrene		193-39-5	Polycyclic aromatic hydrocarbon	
Indole[3,2- <i>b</i>]carbazole			Nitrogen heterocycle	
16 -Iodoestradiol		71765-94-1	Steroid, phenolic	
(<i>E</i>)-17 -Iodovinylestradiol	E-IVE	82123-96-4	Steroid, phenolic	
(<i>Z</i>)-17 -Iodovinylestradiol	Z-IVE	177159-09-0	Steroid, phenolic	
Ipriflavone		35212-22-7	Isoflavone	Pharmaceutical
Isoeugenol	Phenol, 2-methoxy-4-propenyl-	97-54-1	Alkoxyphenol	Natural product; Fragrance; Chemical intermediate (flavor and food additive)
Kaempferol	3,4',5,7-Tetrahydroxyflavone; Kaempherol	520-18-3	Flavone	Natural product
Kepone	Chlordecone	143-50-0	Organochlorine	Pesticide
11-Keto-9 -estradiol			Steroid, phenolic	
16-Ketoestradiol		566-75-6	Steroid, phenolic	
6-Ketoestradiol		571-92-6	Steroid, phenolic	
Lindane	-Hexachlorocyclohexane	58-89-9	Chlorinated cycloalkane; Organochlorine	Pesticide; Pharmaceutical
Melatonin		73-31-4	Aromatic heterocycle	Pharmaceutical
MER-25	Benzeneethanol, -[4-[2-diethylaminoethoxy]phenyl]-4-methoxy- -phenyl-	67-98-1	Alcohol	Pharmaceutical
Mestilbol	Diethylstilbestrol monomethyl ether	18839-90-2	Stilbene	Pharmaceutical
Mestranol	17 alpha-ethylestradiol 3-methyl ether	72-33-3	Steroid, nonphenolic	Pharmaceutical
<i>p,p'</i> -Methoxychlor	(1,1,1-Trichloro-2,2-bis(p-methoxyphenyl)-ethane	72-43-5	Organochlorine	Pesticide
<i>o,p'</i> -Methoxychlor		30667-99-3	Organochlorine	Pesticide
Methyltamoxifen		73617-95-5	Triphenylethylene	
Methyl paraben	Methyl 4-hydroxybenzoate	99-76-3	Paraben	Preservative (foods and cosmetics); Food additive
Methyl methacrylate		80-62-6	Acrylate	Chemical intermediate
Methoxytamoxifen			Triphenylethylene	
Methoxychlor olefin		2132-70-9	Organochlorine	Pesticide
9 -Methylestrone 3-methyl ether		31266-41-8	Steroid, nonphenolic	
9 -Methylestrone		71563-77-4	Steroid, phenolic	
9 -Methylestradiol-17 3-methyl ether		51242-32-1	Steroid, nonphenolic	
9 -Methylestradiol-17		66463-44-3	Steroid, phenolic	
9 -Methyl-14-dehydroestrone 3-methyl ether			Steroid, nonphenolic	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
9 -Methyl-14-dehydroestrone		88598-67-8	Steroid, phenolic	
9 -Methyl-14-dehydroestradiol-17 3-methyl ether		88598-64-5	Steroid, nonphenolic	
9 -Methyl-14-dehydroestradiol-17		88598-63-4	Steroid, phenolic	
7 -Methylestrone 3-methyl ether		10449-00-0	Steroid, nonphenolic	
7 -Methylestrone		10448-96-1	Steroid, phenolic	Pharmaceutical
7 -Methylestradiol-17 3-methyl ether		15506-01-1	Steroid, nonphenolic	
7 -Methylestradiol-17		10448-97-2	Steroid, phenolic	
7 -Methyl-14-dehydroestrone 3-methyl ether		35644-57-6	Steroid, nonphenolic	
7 -Methyl-14-dehydroestrone		88958-66-7	Steroid, phenolic	
7 -Methyl-14-dehydroestradiol-17 3-methyl ether		35644-59-8	Steroid, nonphenolic	
7 -Methyl-14-dehydroestradiol-17		88598-62-3	Steroid, phenolic	
4,4'-Methylenedianiline	Benzenamine, 4,4'-methylenebis- ; 4,4'-Methylenebis(aniline); 4,4'-Methylenebisbenzenamine	101-77-9	Aniline	Chemical intermediate
4,4'-Methylenebis(N,N-dimethylaniline)	4,4'-Methylenebis(N,N-dimethylbenzenamine); Michler's base	101-61-1	Aniline	Chemical intermediate
3-Methoxyestriol	Estriol 3-methyl ether	1474-53-9	Steroid, nonphenolic	
3-Methoxy-10-methyl-11-phenyldibenzo[<i>b,f</i>] thiepin (16)		85807-06-1	Triphenylethylene	
2-(2-Methylphenyl)-3-phenyl-6-hydroxyindene			Triphenylethylene	
1-Methyl-6-hydroxy-2,3-diphenylindene			Triphenylethylene	
1-Methyl-3-ethyl-6,4'-dihydroxy-2-phenylindene			Stilbene	
11 -Methylestrone 3-methyl ether		13667-04-4	Steroid, nonphenolic	
11 -Methylestrone		13667-06-6	Steroid, phenolic	
11 -Methylestradiol-17 3-methyl ether		18046-75-8	Steroid, nonphenolic	
11 -Methylestradiol-17	11 -Methylestradiol	23637-93-6	Steroid, phenolic	
11 -Methyl-14-dehydroestrone 3-methyl ether		88598-69-0	Steroid, nonphenolic	
11 -Methyl-14-dehydroestradiol-17 3-methyl ether		88598-65-6	Steroid, nonphenolic	
(<i>Z</i>)-11 -Methoxy-17 -iodovinylestradiol	Z-MIVE	177159-11-4	Steroid, phenolic	
(<i>E</i>)-11 -Methoxy-17 -iodovinylestradiol	E-MIVE	90857-55-9	Steroid, phenolic	
Metolachlor		51218-45-2	Acetamide; Anilide	Pesticide
Mirex	Dechlorane	2385-85-5	Organochlorine	Pesticide; Fire retardant for plastics, rubber, paint

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Mono- <i>m</i> -acetoxy-1,1,2-triphenylbut-1-ene		82333-69-5	Triphenylethylene	
Monohydroxymethoxychlor		28463-03-8	Organochlorine	Pesticide metabolite
Monohydroxymethoxychlor olefin		75938-34-0	Organochlorine	Pesticide
Monohydroxytamoxifen		68392-35-8	Triphenylethylene	
Morin		480-16-0	Flavone	Dye
Moxestrol	R-2358, 11 -Methoxy-17 -ethinylestradiol	34816-55-2	Steroid, phenolic	Pharmaceutical
Myricetin		529-44-2	Flavone	Natural product
Nafoxidine	Pyrrolidine, 1-(2-(4-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthalenyl)phenoxy)ethyl)-	1845-11-0	Triphenylethylene; Stilbene	Pharmaceutical
Naringenin	4,5,7-Trihydroxyflavanone 7-rhamnoglucoside	480-41-1	Flavanone	
Naringin		10236-47-2	Flavanone	Food additive
Nerolidol		7212-44-4	Terpene	Fragrance
2-Nitroestratriene-3,17 -diol	2-Nitro-1,3,5(10)-estratriene-3,17 -diol	6298-51-7	Steroid, phenolic	
4-Nitroestratriene-3,17 -diol	4-Nitro-1,3,5(10)-estratriene-3,17 -diol; 4-Nitroestradiol	6936-94-3	Steroid, phenolic	
2-Nitroestratrien-3-ol-17-one	2-Nitroestrone	5976-73-8	Steroid, phenolic	
4-Nitroestratrien-3-ol-17-one	4-Nitroestrone	5976-74-9	Steroid, phenolic	
Nitromifene	CN-55,945	10448-84-7	Triphenylethylene	Pharmaceutical
<i>cis</i> -Nonachlor		5103-73-1	Organochlorine; Chlorinated bridged cycloalkene	Pesticide
<i>trans</i> -Nonachlor		39765-80-5	Organochlorine; Chlorinated bridged cycloalkene	Pesticide
Nonylbenzene		1081-77-2	Aromatic hydrocarbon	
<i>p</i> -Nonylphenol	<i>p</i> -Nonylphenol; <i>p-n</i> -Nonylphenol	104-40-5	Phenol; Alkylphenol	Chemical intermediate
<i>n</i> -Nonylphenol		25154-52-3	Phenol; Alkylphenol	Chemical intermediate
Nonylphenol dodecylethoxylate			Phenol; Alkylphenol	Surfactant
Nordihydroguariaretic acid		500-38-9	Bisphenol	Pharmaceutical
Norethindrone		68-22-4	Steroid, nonphenolic	Pharmaceutical
Norethynodrel		68-23-5	Steroid, nonphenolic	Pharmaceutical
19-Nortestosterone		434-22-0	Steroid, nonphenolic	Pharmaceutical
2,2',3,3',4,4',5,5'-Octachlorobiphenyl		35694-08-7	Polychlorinated biphenyl	Dielectric fluid
1,8-Octanediol	1,8-Octamethylenediol	629-41-4	Alcohol	Pharmaceutical
4- <i>n</i> -Octylphenol	4-Octylphenol; <i>p</i> -Octylphenol	1806-26-4	Alkylphenol	Chemical intermediate
4- <i>tert</i> -Octylphenol	<i>p-tert</i> - Octylphenol; 4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	Alkylphenol	Chemical intermediate

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2,2',3,3',6-Pentachlorobiphenyl	PCB 84	52663-60-2	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,5'-Pentachlorobiphenyl	PCB 87	38380-02-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4',6-Pentachlorobiphenyl	PCB 91	68194-05-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3,5',6-Pentachlorobiphenyl	PCB 95	38379-99-6	Polychlorinated biphenyl	Dielectric fluid
2,2',4,4',5-Pentachlorobiphenyl	PCB 99	38380-01-7	Polychlorinated biphenyl	Dielectric fluid
2,2',4,5',5'-Pentachlorobiphenyl	PCB 101	37680-73-2	Polychlorinated biphenyl	Dielectric fluid
2,2',4,6,6'-Pentachlorobiphenyl	PCB 104	56558-16-8	Polychlorinated biphenyl	Dielectric fluid
2,3,3',5,6-Pentachlorobiphenyl	PCB 112	74472-36-9	Polychlorinated biphenyl	Dielectric fluid
2,3,4,4',6-Pentachlorobiphenyl	PCB 115	74472-38-1	Polychlorinated biphenyl	Dielectric fluid
3,3',4,4',5-Pentachlorobiphenyl	PCB 126	57465-28-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3',4',5'-Pentachloro-4-biphenylol		150304-12-4	Polychlorinated biphenyl	
2,2',3',4',6'-Pentachloro-4-biphenylol		150304-10-2	Polychlorinated biphenyl	
2,2',3',5',6'-Pentachloro-4-biphenylol		150304-11-3	Polychlorinated biphenyl	
2,2',4,6,6'-Pentachloro-4-biphenylol			Polychlorinated biphenyl	
2',3,3',4,4'-Pentachloro-2-biphenylol		150975-80-7	Polychlorinated biphenyl	
2,3,3',4',5-Pentachloro-4-biphenylol		152969-11-4	Polychlorinated biphenyl	
2',3,3',4',5-Pentachloro-4-biphenylol		192190-09-3	Polychlorinated biphenyl	
2',3,3',4',5'-Pentachloro-4-biphenylol		149589-55-9	Polychlorinated biphenyl	
2',3,3',4',6'-Pentachloro-4-biphenylol		192190-10-6	Polychlorinated biphenyl	
2',3,3',5',6'-Pentachloro-4-biphenylol		189578-02-7	Polychlorinated biphenyl	
2',3',4,4',5-Pentachloro-3-biphenylol		150975-81-8	Polychlorinated biphenyl	
2,3',4,4',5-Pentachloro-3-biphenylol		170946-11-9	Polychlorinated biphenyl	
2',3,4',5,5'-Pentachloro-4-biphenylol		149589-56-0	Polychlorinated biphenyl	
3,3',4',5,5'-Pentachloro-4-biphenylol		130689-92-8	Polychlorinated biphenyl	
Pentolame		150748-24-6	Steroid, phenolic	
Permethrin	(3-Phenoxyphenyl)methyl (+-) <i>cis,trans</i> -3-(2,2-dichloroethyl)-2,2-dimethyl cyclopropane-carboxylate	52645-53-1	Pyrethrin; Pyrethroid	Pesticide
Phenanthrene		85-01-8	Polycyclic aromatic hydrocarbon	Chemical intermediate
4-Phenethylphenol		6335-83-7	Alkylphenol	
Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis-		107144-81-0	Bisphenol	Chemical intermediate
Phenol, 4-[7-(2-dimethylamino)ethoxy]-11-ethylidibenzo[<i>b,f</i>]thiepin-10-yl]-	3-[2-(Dimethylamino)ethoxy]-10-ethyl-11-(4-hydroxyphenyl)-dibenzo[<i>b,f</i>]thiepin ethyl acetate	85850-74-4	Triphenylethylene	
Phenol, 4-[3-(2-dimethylamino)ethoxy]-11-ethylidibenzo[<i>b,f</i>]thiocin-12-yl)	Phenol, <i>p</i> -[3-(2-(dimethylamino)ethoxy)-11-ethyl-6 <i>H</i> -dibenzo[<i>b,f</i>]thiocin-12-yl)-hydrate	85850-81-3	Triphenylethylene	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Phenol, 4-[2-(2 dimethylamino)-ethoxy]-6-ethyl-11,12-dihydro-dibenzo[<i>a,e</i>]cycloocten-5-yl]-		85850-75-5	Triphenylethylene	
Phenol, 3-[2-dimethylaminoethoxy]-10-ethyl-4-hydroxyphenyl dibenzo[<i>b,f</i>]oxepin	3-[2-(Dimethylamino)ethoxy]-10-ethyl-(4-hydroxyphenyl)-dibenzo[<i>b,f</i>]oxepin	85850-80-2	Triphenylethylene	
Phenol, 4-[1-[4-(2-(dimethylamino)ethoxy]phenyl)-2-phenyl-1-butenyl]-3-methyl-, (<i>E</i>)-	2-Methyl-4-hydroxytamoxifen	96474-35-0	Triphenylethylene	
Phenol, 4-(1, 2-diphenyl-1-butenyl)-		69967-79-9	Triphenylethylene	
Phenol, 4-(1 <i>Z</i>)-1,2-diphenyl-1-butenyl)-		69967-80-2	Triphenylethylene	
Phenol, 4-[2-Nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]ethenyl]phenyl, (<i>E</i>)-		107144-84-3	Triphenylethylene	
Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-		91221-46-4	Triphenylethylene	
Phenolphthalein		77-09-8	Triphenylmethane	Pharmaceutical
Phenolphthalin		81-90-3	Triphenylmethane	Analytical reagent
Phenol Red	Phenolsulfonphthalein; Phenol, 4,4'-(3 <i>H</i> -2,1-benzoxathiol-3-ylidene)di-, <i>S,S</i> -dioxide	143-74-8	Diphenolalkane; Bisphenol	Pharmaceutical
<i>d</i> -Phenothrin		26002-80-2	Pyrethrin; Pyrethroid	Pesticide
2-Phenyl-3-(2-fluoro-4-hydroxyphenyl)-6-hydroxyindene			Indene	
2-Phenyl-3-(2-fluorophenyl)-6-hydroxyindene			Indene	
3-Phenyl-4'-hydroxy-2-phenylindene			Indene	
3-Phenyl-6-hydroxy-2-phenylindene			Indene	
2-Phenyl-3-(2-methylphenyl)-6-hydroxyindene			Indene	
2-Phenyl-3-(4-methylphenyl)-6-hydroxyindene			Indene	
2-Phenylphenol	<i>o</i> -Phenylphenol; 2-Hydroxydiphenyl	90-43-7	Biphenyl; Phenol	Pesticide; Chemical intermediate; Plasticizer; Polymer
3-Phenylphenol		580-51-8	Biphenyl; Phenol	
4-Phenylphenol	<i>p</i> -Phenylphenol; 4-Hydroxybiphenyl	92-69-3	Biphenyl; Phenol	Pesticide; Chemical intermediate; Polymer
Phloretin		60-82-2	Chalconoid	Natural product
Prallethrin		23031-36-9	Pyrethrin; Pyrethroid	Pesticide
Progesterone	Pregn-4-ene-3,20-dione	57-83-0	Steroid, nonphenolic	Pharmaceutical
Prolame		99876-41-2	Steroid, phenolic	
Promegesterone	R5020; 17,21-Dimethyl-19-nor-4,9-pregnadiene-3,20-dione	34184-77-5	Steroid, nonphenolic	Pharmaceutical
Prometon	Prometone; Pramitol; Gesafram 50®; Ontrac800®	1610-18-0	Triazine	Pesticide
Propazine	2-Chloro-4,6-bis(isopropylamino)-s-triazine	139-40-2	Triazine	Pesticide

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Propyl paraben	Propyl 4-hydroxybenzoate; Benzoic acid, <i>p</i> -hydroxy-, propyl ester; Benzoic acid, 4-hydroxy-, propyl ester	94-13-3	Paraben	Pharmaceutical; Preservative (foods); Food additive
Propylpyrazoletriol			Pyrazole	
Prunetin		552-59-0	Isoflavone	Natural product
Pseudodiethylstilbestrol	Pseudo-DES	39011-86-4	Bisphenol; Stilbene	
Pyrene		129-00-0	Polycyclic aromatic hydrocarbon	Dye
Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (<i>E</i>)		77413-87-7	Triphenylethylene	
Quercetin	3,3',4',5,7-Pentahydroxyflavone	117-39-5	Flavone	Natural product (plant)
7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-		107144-83-2		
6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-		107144-82-1		
Raloxifene		84449-90-1	Stilbene; Piperidine; Phenol	Pharmaceutical
Raloxifene hydrochloride	LY 156758	82640-04-8	Nitrogen heterocycle	Pharmaceutical
Resveratrol	3,4',5-Stilbenetriol	501-36-0	Stilbene	Natural product
Rutin		153-18-4	Flavone	Natural product
Simazine	s-Triazine, 2-chloro-4,6-bis(ethylamino)- ; 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl-	122-34-9	Organochlorine; Triazine	Pesticide
-Sitosterol	24-alpha-Ethylcholesterol	83-46-5	Steroid, nonphenolic	Natural product (animal); Pharmaceutical
4,4'-Stilbenediol	4,4'-Dihydroxystilbene; Phenol, 4,4'-(1,2-ethenediyl)bis-	659-22-3	Stilbene; Bisphenol	
4-Stilbenol		3839-46-1	Stilbene; Phenol	
Suberic acid	Octanedioic acid	505-48-6	Carboxylic acid	Chemical intermediate
Tamoxifen	ICI 47699	10540-29-1	Stilbene	Pharmaceutical
Tamoxifen citrate		54965-24-1	Stilbene	Pharmaceutical
Taxifolin		480-18-2	Flavanone	Pharmaceutical
Testosterone	Androst-4-en-3-one, 17-hydroxy-, (17)-	58-22-0	Steroid, nonphenolic	Pharmaceutical
2,2',3,3'-Tetrachlorobiphenyl	PCB 44	3844-93-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4'-Tetrachlorobiphenyl	PCB 41	52663-59-9	Polychlorinated biphenyl	Dielectric fluid
2,2',3,6'-Tetrachlorobiphenyl	PCB 45	41464-47-5	Polychlorinated biphenyl	Dielectric fluid
2,2',4,4'-Tetrachlorobiphenyl	PCB 47	2437-79-8	Polychlorinated biphenyl	Dielectric fluid
2,2',4,5'-Tetrachlorobiphenyl	PCB 49	41464-40-8	Polychlorinated biphenyl	Dielectric fluid
2,2',4,6'-Tetrachlorobiphenyl	PCB 51	68194-04-7	Polychlorinated biphenyl	Dielectric fluid
2,2',5,5'-Tetrachlorobiphenyl	PCB 52	35693-99-3	Polychlorinated biphenyl	Dielectric fluid

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2,2',6,6'-Tetrachlorobiphenyl	PCB 54	15968-05-5	Polychlorinated biphenyl	Dielectric fluid
2,3,3',5'-Tetrachlorobiphenyl	PCB 58	41464-49-7	Polychlorinated biphenyl	Dielectric fluid
2,3,4,4'-Tetrachlorobiphenyl	PCB 60	33025-41-1	Polychlorinated biphenyl	Dielectric fluid
2,3',4,5'-Tetrachlorobiphenyl	PCB 68	73575-52-7	Polychlorinated biphenyl	Dielectric fluid
2,3',4',5-Tetrachlorobiphenyl	PCB 70	32598-11-1	Polychlorinated biphenyl	Dielectric fluid
2,4,4',5-Tetrachlorobiphenyl	PCB 74	32690-93-0	Polychlorinated biphenyl	Dielectric fluid
3,3',4,4'-Tetrachlorobiphenyl	PCB 77	32598-13-3	Polychlorinated biphenyl	Dielectric fluid
3,3',4,5-Tetrachlorobiphenyl	PCB 78	70362-49-1	Polychlorinated biphenyl	Dielectric fluid
2',3',5',6'-Tetrachloro-4,4'-biphenyldiol		100702-98-5	Polychlorinated biphenyl	
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol		13049-13-3	Polychlorinated biphenyl	
2,2',4',6'-Tetrachloro-4-biphenylol		150304-08-8	Polychlorinated biphenyl	
2,2',6,6'-Tetrachloro-4-biphenylol		219952-18-8	Polychlorinated biphenyl	
2',3',4',5'-Tetrachloro-3-biphenylol		67651-37-0	Polychlorinated biphenyl	
2',3',4',5'-Tetrachloro-4-biphenylol		67651-34-7	Polychlorinated biphenyl	
2',3,4',6'-Tetrachloro-4-biphenylol		189578-00-5	Polychlorinated biphenyl	
2,3,7,8-Tetrachlorodibenzo-p-dioxin	Dioxin; 2,3,7,8-TCDD	1746-01-6	Dioxin	
Tetrahydrochrysene		104460-72-2	Polycyclic aromatic hydrocarbon	
(<i>R,R</i>) -Tetrahydrochrysene			Polycyclic aromatic hydrocarbon	
(<i>S,S</i>) -Tetrahydrochrysene			Polycyclic aromatic hydrocarbon	
2,2',4,4'-Tetrahydroxybenzil		5394-98-9	Benzophenone	
Tetramethylhexestrol		74385-27-6	Stilbene	
Thalidomide		50-35-1	Phthalimide	Pharmaceutical
Toremifene citrate		89778-27-8	Triphenylethylene	Pharmaceutical
Tosyl nonylphenol (mixed branched isomers)			Phenol; Alkylphenol	
Toxaphene		8001-35-2	Organochlorine	Pesticide
Triaryl-pyrazole				
2,2',5-Trichlorobiphenyl	PCB 18	37680-65-2	Polychlorinated biphenyl	Dielectric fluid
2,4,6-Trichlorobiphenyl	PCB 30	35693-92-6	Polychlorinated biphenyl	Dielectric fluid
2',4',6'-Trichloro-4-biphenylol		14962-28-8	Polychlorinated biphenyl	
3,3',4-Trichloro-4-biphenylol		124882-64-0	Polychlorinated biphenyl	
3,4',5-Trichloro-4-biphenylol		4400-06-0	Polychlorinated biphenyl	
2,4,5-Trichlorophenoxyacetic acid	2,4,5-T acid; Esterone 245; Trioxone; Trichlorophenoxyacetic acid	93-76-5	Chlorinated aromatic hydrocarbon; Organochlorine	Pesticide

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Triethylamine, 2-[p -[6-methoxy-2-phenyl-3-inden-3-yl)phenoxy]hydrochloride	U-11555A	64-96-0	Triphenylethylene	
Triethylene glycol dimethacrylate		109-16-0	Acrylate	Plasticizer
4,2',4'-Trihydroxychalcone		961-29-5	Chalconoid	
3,6,4',-Trihydroxyflavone		253195-19-6	Flavone	
6,7,4'-Trihydroxyisoflavone		17817-31-1	Isoflavone	
7,3',4'-Trihydroxyisoflavone		485-63-2	Isoflavone	
1,1,2-Triphenylbut-1-ene		63019-13-6	Triphenylethylene	
Triphenylethylene		58-72-0	Triphenylethylene	
Triphenyl phosphate	Phosphoric acid, triphenyl ester	115-86-6	Phosphate ester	Plasticizer
Tris(4-chlorophenyl)methane	Tris-H	27575-78-6	Organochlorine	
Tris(4-chlorophenyl)methanol	Tris-OH	30100-80-8	Organochlorine	
Vanillin	4-Hydroxy-3-methoxybenzaldehyde	121-33-5	Alkoxyphenol	Flavor; Chemical intermediate; Pharmaceutical (additive); Food additive
Vinclozolin		50471-44-8	Organochlorine	Pesticide
- Zearalanol		26538-44-3	Resorcylic acid lactone; Phenol	Natural product
- Zearalanol		42422-68-4	Resorcylic acid lactone	Natural product
Zearalanone		5975-78-0	Resorcylic acid lactone	Natural product
-Zearalenol		36455-72-8	Resorcylic acid lactone; Phenol	Natural product
Zearalenone		17924-92-4	Resorcylic acid lactone; Phenol	Natural product
- Zearalenol		71030-11-0	Resorcylic acid lactone; Phenol	Natural product

Appendix D

Substances Tested in the *In Vitro* ER Binding Assays

D1 Data Sorted by Substance Name and Assay

D2 References

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Appendix D1

Substances Tested in the *In Vitro* ER Binding Assays

Data Sorted by Substance Name and Assay

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Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1											
2	hER -FP	4,4'-(1,3-Adamantanediyl)diphenol			0.2	0.001			6.5	0.813	Nikov et al. (2001)
3	hER -FP	2-(1-Adamantyl)-4-methylphenol	41031-50-9				10				Nikov et al. (2001)
4	hER -FP	4-(1-Adamantyl)phenol	29799-07-3		1	1			1.3	0.114	Nikov et al. (2001)
5	hER	Alachlor	15972-60-8				50				Klotz et al. (1996)
6	RUC	Alachlor	15972-60-8	98.8			100				Blair et al. (2000)
7	RUC	Aldosterone	52-39-1	98			100				Blair et al. (2000)
8	RUC	Aldrin	309-00-2	98			600				Blair et al. (2000)
9	hER -FP	Allethrin	584-79-2	> 93			10				Saito et al. (2000)
10	RUC	<i>p</i> -(7-Alloxy)-11-ethyl-dibenzo- <i>b,f</i>]thiepin-10-yl)phenol	85850-86-8						5.2	0.716	Acton et al. (1983)
11	RUC	<i>p</i> -(3-(Alloxy)-11-ethyl-6 <i>H</i> -dibenzo[<i>b,f</i>]thiocin-12-yl)phenol hemihydrate	85850-88-0						9.2	0.964	Acton et al. (1983)
12	RUC	<i>p</i> -(2-(Alloxy)-6-ethyl-11,12-dihydroxydibenzo[<i>a,e</i>]cyclooctene-5-yl)phenol	85850-87-9						15	1.176	Acton et al. (1983)
13	RUC	3-(Alloxy)-10-ethyl-11-(4-hydroxyphenyl)dibenzo[<i>b,f</i>]thiepin	85850-85-7						0.21	-0.678	Acton et al. (1983)
14	RUC	3-(Alloxy)-10-ethyl-11-phenyldibenzo[<i>b,f</i>]thiepin	85850-82-4						0.54	-0.268	Acton et al. (1983)
15	RUC	3-(Alloxy)-11-ethyl-12-phenyl 6 <i>H</i> -dibenzo[<i>b,f</i>]thiocin	85850-84-6						0.12	-0.921	Acton et al. (1983)
16	RUC	3-(Alloxy)-10-ethyl-11-phenyldibenzo[<i>b,f</i>]joxepin	83807-07-2						0.1	-1.000	Acton et al. (1983)
17	RUC	3-(Alloxy)-11-ethyl-12-phenyl 5,6-dihydroxydibenzo-[<i>a,e</i>]cyclooctene	85850-83-5						0.36	-0.444	Acton et al. (1983)
18	RUC	Amaranth	915-67-3	80			100				Blair et al. (2000)
19	MCF-7 cytosol	2-Aminoestratriene-3,17 -diol	107900-30-1						12	1.079	Brooks et al. (1987)
20	MCF-7 cytosol	4-Aminoestratriene-3,17 -diol	107900-31-2						16	1.204	Brooks et al. (1987)
21	MCF-7 cytosol	2-Aminoestratrien-17 -ol	17522-06-4						4	0.602	Brooks et al. (1987)
22	MCF-7 cytosol	4-Aminoestratrien-17 -ol	17522-04-2						0.17	-0.770	Brooks et al. (1987)
23	RUC	4-Aminophenyl ether	101-80-4	99			1000				Blair et al. (2000)
24	hER	4- <i>tert</i> -Amylphenol	80-46-6				10				Kuiper et al. (1998) [method a]
25	hER	4- <i>tert</i> -Amylphenol	80-46-6				10				Kuiper et al. (1998) [method a]
26	RUC	4- <i>tert</i> -Amylphenol	80-46-6	99	165	45			0.0005	-3.260	Blair et al. (2000)
27	hER	3 -Androstenediol	25126-76-5					0.006	3	0.477	Kuiper et al. (1997)
28	rER	3 -Androstenediol	25126-76-5					0.002	7	0.845	Kuiper et al. (1997)
29	MUC	5 -Androstane-3 ,17 -diol	1852-53-5				10				Korach (1979)
30	RUC	5 -Androstane-3 ,17 -diol	1852-53-5	99	42	1.6			0.002	-2.670	Blair et al. (2000)
31	MCF-7 cytosol	5 -Androstane-3 ,17 -diol	1852-53-5				0.1				VanderKuur et al. (1993)
32	hER	5 -Androstane-3 ,17 -diol	571-20-0					0.26	0.07	-1.150	Kuiper et al. (1997)
33	MUC	5 -Androstane-3 ,17 -diol	571-20-0						0.5	-0.300	Korach (1979)
34	rER	5 -Androstane-3 ,17 -diol	571-20-0					0.048	0.3	-0.523	Kuiper et al. (1997)
35	MCF-7 cytosol	5 -Androstane-3 ,17 -diol	571-20-0						0.005	-2.301	VanderKuur et al. (1993)
36	RUC	5 -Androstane-3 ,17 -diol	571-20-0		0.75	0.13			0.12	-0.920	Blair et al. (2000)
37	MUC	5 -Androstane-3 ,17 -diol	1851-23-6				10				Korach (1979)
38	hER	5 -Androstenedione	1229-12-5				100				Kuiper et al. (1997)
39	rER	5 -Androstenedione	1229-12-5				100				Kuiper et al. (1997)
40	hER	5 -Androstane-3,17-dione	5982-99-0				100				Kuiper et al. (1997)
41	MUC	5 -Androstane-3,17-dione	5982-99-0				10				Korach (1979)
42	rER	5 -Androstane-3,17-dione	5982-99-0				100				Kuiper et al. (1997)
43	MUC	5 -Androstane-3 -ol-17-one	53-41-8				10				Korach (1979)
44	hER	4-Androstenediol	1156-92-9					0.023	0.5	-0.300	Kuiper et al. (1997)
45	rER	4-Androstenediol	1156-92-9					0.019	0.6	-0.222	Kuiper et al. (1997)
46	hER	5-Androstenediol	521-17-5					0.0036	6	0.778	Kuiper et al. (1997)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
47	hER	5-Androstenediol	521-17-5						1	0.000	Kuiper et al. (1998) [method a]
48	hER	5-Androstenediol	521-17-5		0.024				3.9	0.590	Kuiper et al. (1998) [method b]
49	hER	5-Androstenediol	521-17-5						7	0.845	Kuiper et al. (1998) [method a]
50	hER	5-Androstenediol	521-17-5		0.005				21.2	1.330	Kuiper et al. (1998) [method b]
51	RBC	5-Androstenediol	521-17-5						1	0.000	Korenman (1969)
52	rER	5-Androstenediol	521-17-5					0.0009	17	1.230	Kuiper et al. (1997)
53	hER	4-Androstenedione	63-05-8				100				Kuiper et al. (1997)
54	MCF-7 cytosol	4-Androstenedione	63-05-8						0.007	-2.155	VanderKuur et al. (1993)
55	MUC	4-Androstenedione	63-05-8				10				Korach (1979)
56	rER	4-Androstenedione	63-05-8				100				Kuiper et al. (1997)
57	MCF-7 cells	Anthracene	120-12-7				5				Arcaro et al. (1999)
58	hER	Apigenin	520-36-5						0.3	-0.523	Kuiper et al. (1998) [method a]
59	hER	Apigenin	520-36-5						6	0.778	Kuiper et al. (1998) [method a]
60	hER	Apigenin	520-36-5		0.058				2	0.301	Kuiper et al. (1998) [method b]
61	RUC	Apigenin	520-36-5						0.028	-0.620	Fang et al. (2001)
62	RUC	Aroclor 1221	11104-28-2				100 *				Nelson (1974)
63	RUC	Aroclor 1254	11097-69-1				100				Nelson (1974)
64	GST-aERdef	Atrazine	1912-24-9				100				Matthews et al. (2000)
65	GST-cERdef	Atrazine	1912-24-9				100				Matthews et al. (2000)
66	GST-hER def	Atrazine	1912-24-9				100				Matthews et al. (2000)
67	GST-mER def	Atrazine	1912-24-9				100				Matthews et al. (2000)
68	GST-rtERdef	Atrazine	1912-24-9				100				Matthews et al. (2000)
69	hER -FP	Atrazine	1912-24-9	99.1			2000				Hanioka et al. (1999)
70	RUC	Atrazine	1912-24-9	98			100				Blair et al. (2000)
71	RUC	Atrazine	1912-24-9					1000	0.0003	-3.523	Waller et al. (1996)
72	RUC	Aurin	603-45-2		2.8	1.8			0.032	-1.490	Blair et al. (2000)
73	RUC	Baicalein	491-67-8						0.0009	-3.046	Fang et al. (2001)
74	hER	Benomyl	17804-35-2				50				Klotz et al. (1996)
75	GST-hER def	Benzo[a]anthracene	56-55-3				10				Fertuck et al. (2001)
76	hER	Benzo[a]anthracene	56-55-3				10				Fertuck et al. (2001)
77	MCF-7 cells	Benzo[a]anthracene	56-55-3						33	1.519	Arcaro et al. (1999)
78	MCF-7 cells	Benzenecetonitrile -[bis(4-hydroxyphenyl) methylene]	66422-14-8						8.5	0.929	Stoessel and Leclercq (1986)
79	MCF-7 cytosol	Benzenecetonitrile -[bis(4-hydroxyphenyl) methylene]	66422-14-8						100	2.000	Stoessel and Leclercq (1986)
80	GST-hER def	Benzo[a]carbazole	239-01-0				10				Fertuck et al. (2001)
81	hER	Benzo[a]carbazole	239-01-0				10				Fertuck et al. (2001)
82	GST-hER def	Benzo[c]carbazole					10				Fertuck et al. (2001)
83	hER	Benzo[c]carbazole					10				Fertuck et al. (2001)
84	MCF-7 cells	Benzo[b]fluoranthene	205-99-2						17	1.230	Arcaro et al. (1999)
85	MCF-7 cells	Benzo[k]fluoranthene	207-08-9						27	1.431	Arcaro et al. (1999)
86	RUC	Benzo[a]fluorene	238-84-6	98			33.3				Blair et al. (2000)
87	GST-hER def	Benzo[b]fluorene	243-17-4				10				Fertuck et al. (2001)
88	hER	Benzo[b]fluorene	243-17-4				10				Fertuck et al. (2001)
89	GST-hER def	Benzo[b]naphtho[2,1-d]thiophene	239-35-0				10				Fertuck et al. (2001)
90	hER	Benzo[b]naphtho[2,1-d]thiophene	239-35-0				10				Fertuck et al. (2001)
91	GST-hER def	Benzo[b]naphtho[2,3-d]thiophene	243-46-9				10				Fertuck et al. (2001)
92	hER	Benzo[b]naphtho[2,3-d]thiophene	243-46-9				10				Fertuck et al. (2001)
93	MCF-7 cells	Benzo[ghi]perylene	191-24-2				5				Arcaro et al. (1999)
94	GST-hER def	Benzo[c]phenanthrene	195-19-7				10				Fertuck et al. (2001)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1											
95	hER	Benzo[<i>c</i>]phenanthrene	195-19-7				10				Fertuck et al. (2001)
96	MCF-7 cells	Benzo[<i>a</i>]pyrene	50-32-8						14	1.146	Arcaro et al. (1999)
97	MCF-7 cells	Benzo[<i>e</i>]pyrene	192-97-2						57	1.756	Arcaro et al. (1999)
98	RUC	Benzyl alcohol	100-51-6	99.7			10000				Blair et al. (2000)
99	RUC	4-Benzylloxyphenol	103-16-2	99	250	50			0.00036	-3.440	Blair et al. (2000)
100	RUC	Benzylparaben	94-18-8	99	31.5	3.5			0.003	-2.540	Blair et al. (2000)
101	hER	Biochanin A	491-80-5				10				Kuiper et al. (1998) [method a]
102	hER	Biochanin A	491-80-5				10				Kuiper et al. (1998) [method a]
103	RUC	Biochanin A	491-80-5						0.0043	-2.370	Fang et al. (2001)
104	RUC	Bis(<i>m</i> -acetoxy)-1,1,2-triphenylbut-1-ene	100808-56-8						12	1.079	Jordan et al. (1986)
105	RUC	Bis(<i>p</i> -acetoxy)-1,1,2-triphenylbut-1-ene	100808-54-6						73	1.863	Jordan et al. (1986)
106	RUC	Bisdexoyestradiol	1217-09-0		5				0.1	-1.000	Elsby et al. (2000)
107	RUC	1,1-Bis-(4-hydroxyphenyl) ethane	2081-08-5	97					0.0009	-3.046	Perez et al. (1998)
108	RUC	4,4-Bis(4-hydroxyphenyl) heptane	7425-79-8	97					0.15	-0.824	Perez et al. (1998)
109	MCF-7 cells	3,4-Bis(3-hydroxyphenyl)- hexane	68266-24-0						20	1.301	Stoessel and Leclercq (1986)
110	MCF-7 cytosol	3,4-Bis(3-hydroxyphenyl)- hexane	68266-24-0						10	1.000	Stoessel and Leclercq (1986)
111	RUC	3,3-Bis(4-hydroxyphenyl) pentane	3600-64-4	97					0.18	-0.745	Perez et al. (1998)
112	RUC	1,1-Bis(4-hydroxyphenyl) propane	1576-13-2	97					0.15	-0.824	Perez et al. (1998)
113	RUC	2,2-Bis(4-hydroxyphenyl) propanol	142648-65-5	97					0.0075	-2.125	Perez et al. (1998)
114	GST-aERdef	(2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.064	0.017			4.8	0.681	Matthews et al. (2000)
115	GST-cERdef	(2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.068	0.022			4.8	0.681	Matthews et al. (2000)
116	GST-mER def	(2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.22	0.02			1.2	0.079	Matthews et al. (2000)
117	GST-rtERdef	(2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.024	0.001			14	1.146	Matthews et al. (2000)
118	hER -FP	(2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	99	0.75				1.7	0.230	Bolger et al. (1998)
119	GST-hER def	(2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.25	0.08			1.2	0.079	Matthews et al. (2000)
120	hER	2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	> 97	1				0.4	-0.398	Gaido et al. (1999)
121	hER	2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	> 97					2	0.301	Gaido et al. (1999)
122	MUC	2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	> 99	0.15				1.2	0.079	Shelby et al. (1996)
123	RUC	2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0					0.05	5.2	0.716	Waller et al. (1996)
124	RUC	2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	98	0.355	0.015			0.25	-0.600	Blair et al. (2000)
125	RUC	2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.141			0.053	0.75	-0.122	Laws et al. (2000)
126	GST-aERdef	Bisphenol A	80-05-7		2.4	1.6			0.13	-0.886	Matthews et al. (2000)
127	GST-cERdef	Bisphenol A	80-05-7		7.3	1.9			0.044	-1.357	Matthews et al. (2000)
128	GST-hER def	Bisphenol A	80-05-7		36	16			0.008	-2.097	Matthews et al. (2000)
129	GST-mER def	Bisphenol A	80-05-7		31	7			0.0086	-2.066	Matthews et al. (2000)
130	GST-rtERdef	Bisphenol A	80-05-7		1.6	0.3			0.21	-0.678	Matthews et al. (2000)
131	hER	Bisphenol A	80-05-7					0.20	0.05	-1.300	Kuiper et al. (1997)
132	hER	Bisphenol A	80-05-7						0.01	-2.000	Kuiper et al. (1998) [method a]
133	hER	Bisphenol A	80-05-7		150				0.003	-2.48	Morito et al. (2001)
134	hER -FP	Bisphenol A	80-05-7	99	32				0.04	-1.398	Bolger et al. (1998)
135	hER -FP	Bisphenol A	80-05-7	> 99	100				0.01	-2.000	Hashimoto et al. (2000)
136	hER	Bisphenol A	80-05-7						0.01	-2.000	Kuiper et al. (1998) [method a]
137	hER	Bisphenol A	80-05-7		8				0.063	-1.20	Morito et al. (2001)
138	MCF-7 cells	Bisphenol A	80-05-7	100					0.006	-2.222	Nagel et al. (1997)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
139	MCF-7 cytosol	Bisphenol A	80-05-7		0.9				1	0.000	Dodge et al. (1996)
140	MUC	Bisphenol A	80-05-7	99.9	26	11			0.01	-1.939	Matthews et al. (2001)
141	RBC	Bisphenol A	80-05-7	99	1.6				0.0013	-2.886	Andersen et al. (1999)
142	rER	Bisphenol A	80-05-7					0.035	0.33	-0.481	Kuiper et al. (1997)
143	RUC	Bisphenol A	80-05-7	99	4.192			1.57	0.026	-1.590	Laws et al. (2000)
144	RUC	Bisphenol A	80-05-7		10				0.12	-0.921	Olea et al. (1996)
145	RUC	Bisphenol A	80-05-7					1.45	0.1793	-0.746	Waller et al. (1996)
146	RUC	Bisphenol A	80-05-7	99	11.7	6.4			0.008	-2.110	Blair et al. (2000)
147	RUC	Bisphenol A	80-05-7						0.056	-1.252	Perez et al. (1998)
148	RUC	Bisphenol A bis(chloroformate)	2024-88-6						0.023	-1.638	Perez et al. (1998)
149	RUC	Bisphenol A diglycidyl ether	1675-54-3				100				Olea et al. (1996)
150	RUC	Bisphenol A diglycidyl ether	1675-54-3				200				Perez et al. (1998)
151	hER -FP	Bisphenol A diglycidyl ether dimethacrylate	1565-94-2	99			5000				Hashimoto et al. (2000)
152	RUC	Bisphenol A diglycidyl ether dimethacrylate	1565-94-2				100				Olea et al. (1996)
153	RUC	Bisphenol A diglycidyl ether dimethacrylate	1565-94-2				200				Perez et al. (1998)
154	RBC	Bisphenol A dimethacrylate	3253-39-2	99.7	4.3				0.00047	-3.328	Andersen et al. (1999)
155	RUC	Bisphenol A dimethacrylate	3253-39-2		300				0.033	-1.481	Olea et al. (1996)
156	RUC	Bisphenol A dimethacrylate	3253-39-2						0.0015	-2.824	Perez et al. (1998)
157	RUC	Bisphenol A ethoxylate	68140-85-2				200				Perez et al. (1998)
158	RUC	Bisphenol A ethoxylate diacrylate	64401-02-1						0.0005	-3.301	Perez et al. (1998)
159	MUC	Bisphenol A glucuronide					100				Matthews et al. (2001)
160	RUC	Bisphenol A propoxylate	37353-75-6				200				Perez et al. (1998)
161	RUC	Bisphenol AF	1478-61-1	97					1	0.000	Perez et al. (1998)
162	RUC	Bisphenol B	77-40-7		1.05	0.46			0.086	-1.070	Blair et al. (2000)
163	RUC	Bisphenol B	77-40-7	97					0.15	-0.824	Perez et al. (1998)
164	RUC	Bisphenol C	79-97-0	97					0.25	-0.602	Perez et al. (1998)
165	MCF-7 cells	Bisphenol C 2	14868-03-2						0.3	-0.523	Stoessel and Leclercq (1986)
166	MCF-7 cytosol	Bisphenol C 2	14868-03-2						2	0.301	Stoessel and Leclercq (1986)
167	RUC	Bisphenol C 2	14868-03-2	98	0.034	0.004			2.64	0.420	Blair et al. (2000)
168	RUC	Bisphenol E	6052-84-2		2.45	0.35			0.037	-1.440	Blair et al. (2000)
169	RUC	2,2'-Bisphenol F	2467-02-9	98			10				Blair et al. (2000)
170	RUC	4,4'-Bisphenol F	620-92-8	98	95	5			0.0009	-3.020	Blair et al. (2000)
171	RUC	4,4'-Bisphenol F	620-92-8						0.15	-0.824	Perez et al. (1998)
172	RUC	Bisphenol S	80-09-1	99	105	35			0.0009	-3.070	Blair et al. (2000)
173	hER	16 -Bromo-17 -estradiol	54982-79-5						76	1.881	Kuiper et al. (1998) [method a]
174	hER	16 -Bromo-17 -estradiol	54982-79-5		0.00172				54.0	1.730	Kuiper et al. (1998) [method b]
175	hER	16 -Bromo-17 -estradiol	54982-79-5						10	1.000	Kuiper et al. (1998) [method a]
176	hER	16 -Bromo-17 -estradiol	54982-79-5		0.0063				16.8	1.230	Kuiper et al. (1998) [method b]
177	MCF-7 cells	1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl)phenoxy]-	107144-85-4						7	0.845	Stoessel and Leclercq (1986)
178	MCF-7 cytosol	1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl)phenoxy]-	107144-85-4						30	1.477	Stoessel and Leclercq (1986)
179	MCF-7 cells	1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthenyl)-phenoxy]-	107163-56-4						0.06	-1.222	Stoessel and Leclercq (1986)
180	MCF-7 cytosol	1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthenyl)-phenoxy]-	107163-56-4						0.1	-1.000	Stoessel and Leclercq (1986)
181	RUC	Butolame	150748-23-5		6				0.14	-0.854	Jaimez et al. (2000)
182	RUC	Butyl 4-aminobenzoate	94-25-7	99			100				Blair et al. (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
183	RUC	<i>n</i> -Butylbenzene	104-51-8	99			200				Blair et al. (2000)
184	RUC	<i>sec</i> -Butylbenzene	135-98-8	99			1000				Blair et al. (2000)
185	GST-aERdef	Butyl benzyl phthalate	85-68-7				100				Matthews et al. (2000)
186	GST-cERdef	Butyl benzyl phthalate	85-68-7				100				Matthews et al. (2000)
187	GST-hER def	Butyl benzyl phthalate	85-68-7				100				Matthews et al. (2000)
188	GST-mER def	Butyl benzyl phthalate	85-68-7				100				Matthews et al. (2000)
189	GST-rtERdef	Butyl benzyl phthalate	85-68-7				100				Matthews et al. (2000)
190	hER -FP	Butyl benzyl phthalate	85-68-7	98	105				0.012	-1.921	Bolger et al. (1998)
191	hER -FP	Butyl benzyl phthalate	85-68-7	97	5000				0.0002	-3.699	Hashimoto et al. (2000)
192	RBC	Butyl benzyl phthalate	85-68-7	97			100				Andersen et al. (1999)
193	RUC	Butyl benzyl phthalate	85-68-7	98			1000				Blair et al. (2000)
194	RUC	Butyl benzyl phthalate	85-68-7				500				Elsby et al. (2000)
195	RUC	Butyl benzyl phthalate	85-68-7				100				Fang et al. (2001)
196	RUC	Butyl benzyl phthalate	85-68-7					76.38	0.0034	-2.470	Waller et al. (1996)
197	RUC	Butyl benzyl phthalate	85-68-7	98.5	36				0.000036	-4.444	Zacharewski et al. (1998)
198	RUC	Butylparaben	94-26-8	99	105	35			0.0009	-3.07	Blair et al. (2000)
199	RUC	Butylparaben	94-26-8	> 99	10				0.002	-2.699	Routledge et al. (1998)
200	RUC	2- <i>sec</i> -Butylphenol	89-72-5	98	315	5			0.00029	-3.540	Blair et al. (2000)
201	RUC	2- <i>tert</i> -Butylphenol	88-18-6					232	0.0011	-2.959	Waller et al. (1996)
202	RUC	3- <i>tert</i> -Butylphenol	585-34-2					395	0.0007	-3.155	Waller et al. (1996)
203	RUC	4- <i>sec</i> -Butylphenol	99-71-8	96	210	30			0.00043	-3.37	Blair et al. (2000)
204	hER	4- <i>tert</i> -Butylphenol	98-54-4				10				Kuiper et al. (1998) [method a]
205	hER	4- <i>tert</i> -Butylphenol	98-54-4				10				Kuiper et al. (1998) [method a]
206	RUC	4- <i>tert</i> -Butylphenol	98-54-4					161	0.0016	-2.796	Waller et al. (1996)
207	RUC	4- <i>tert</i> -Butylphenol	98-54-4	99	368	83			0.00024	-3.610	Blair et al. (2000)
208	hER -FP	Butyl phthalyl <i>n</i> -butyl glycolate	85-70-1	93			5000				Hashimoto et al. (2000)
209	RUC	Caffeine	58-08-2	100			100				Blair et al. (2000)
210	RUC	Carbaryl	63-25-2	99			100				Blair et al. (2000)
211	RUC	Carbofuran	1563-66-2	98			100				Blair et al. (2000)
212	RUC	Castor oil	8001-79-4				100				Blair et al. (2000)
213	RUC	(±)-Catechin	7295-85-4				100				Fang et al. (2001)
214	RUC	Chalcone	94-41-7						0.0015	-2.820	Fang et al. (2001)
215	hER	Chlordane	57-74-9	95 - 99			10				Arcaro et al. (2000)
216	RUC	-Chlordane	5103-71-9				1000				Blair et al. (2000)
217	RBC	Chlormequat chloride	999-81-5	97			100				Andersen et al. (1999)
218	hER -FP	2-Chloro-4-amino-6-isopropylamino-1,3,5-triazine	6190-65-4	99.7	951	105			0.00002	-4.699	Hanioka et al. (1999)
219	MUC	2'-Chloro-4,4'-biphenyldiol	56858-70-9	> 98	0.0900				1.11	0.045	Korach et al. (1988)
220	MUC	2-Chloro-4-biphenylol	23719-22-4	> 98	2.50				0.040	-1.398	Korach et al. (1988)
221	RUC	2-Chloro-4-biphenylol	23719-22-4	95	52.5	25.5			0.002	-2.770	Blair et al. (2000)
222	MUC	4-Chloro-4'-biphenylol	28034-99-3	> 98	3.9				0.026	-1.585	Korach et al. (1988)
223	MUC	4-Chloro-4'-biphenylol	28034-99-3					5.57	0.047	-1.330	Waller et al. (1996)
224	RUC	4-Chloro-4'-biphenylol	28034-99-3	95	13.5	1.5			0.007	-2.180	Blair et al. (2000)
225	RUC	4-Chloro- <i>m</i> -cresol	59-50-7	99	215	15			0.00042	-3.380	Blair et al. (2000)
226	RUC	2-Chloro-4,6-diamino-5' -triazine	3397-62-4					1000	0.0003	-3.523	Waller et al. (1996)
227	hER -FP	2-Chloro-4-ethylamino-6-amino-1,3,5-triazine	1007-28-9	99.2			2000				Hanioka et al. (1999)
228	hER -FP	2-Chloro-4-ethylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine	142179-80-4	99.5			2000				Hanioka et al. (1999)
229	hER -FP	2-Chloro-4-isopropylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine	142200-36-0	99.1			2000				Hanioka et al. (1999)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
230	MCF-7 cells	11 -Chloromethylestradiol	71794-60-0						96	1.982	Stoessel and Leclerq (1986)
231	MCF-7 cytosol	11 -Chloromethylestradiol	71794-60-0						100	2.000	Stoessel and Leclerq (1986)
232	RUC	2-Chloro-4-methylphenol	6640-27-3	97	415	175			0.00022	-3.660	Blair et al. (2000)
233	RUC	4-Chloro-2-methylphenol	1570-64-5	97	425	105			0.00021	-3.670	Blair et al. (2000)
234	RUC	2-Chlorophenol	95-57-8	99			200				Blair et al. (2000)
235	RUC	4-Chlorophenol	106-48-9	90	25.5	1.5			0.004	-2.450	Blair et al. (2000)
236	RUC	Chlorotamoxifen	77588-46-6				10				Allen et al. (1980)
237	RUC	Cholesterol	57-88-5	99			1000				Blair et al. (2000)
238	GST-hER def	Chrysene	218-01-9				10				Fertuck et al. (2001)
239	hER	Chrysene	218-01-9				10				Fertuck et al. (2001)
240	MCF-7 cells	Chrysene	218-01-9						13	1.114	Arcaro et al. (1999)
241	RUC	Chrysene	218-01-9	98			10				Blair et al. (2000)
242	hER	Chrysin	480-40-0				10				Kuiper et al. (1998) [method a]
243	hER	Chrysin	480-40-0				10				Kuiper et al. (1998) [method a]
244	RUC	Chrysin	480-40-0				100				Fang et al. (2001)
245	RUC	Cineole	470-82-6	90			10000				Blair et al. (2000)
246	RUC	Cinnamic acid	621-82-9	99.9			1000				Blair et al. (2000)
247	RBC	cis -Clomiphene	15690-55-8						0.12	-0.910	Korenman (1970)
248	hER	trans -Clomiphene	911-45-5					0.0009	25	1.398	Kuiper et al. (1997)
249	RBC	trans -Clomiphene	911-45-5						0.0081	-2.092	Korenman (1970)
250	rER	trans -Clomiphene	911-45-5					0.0012	12	1.079	Kuiper et al. (1997)
251	RUC	Clomiphene citrate	50-41-9	98	0.125	0.075			0.72	-0.140	Blair et al. (2000)
252	RBC	Colchicine	64-86-8	95			100				Andersen et al. (1999)
253	hER	Corticosterone	50-22-6				100				Kuiper et al. (1997)
254	rER	Corticosterone	50-22-6				100				Kuiper et al. (1997)
255	RUC	Corticosterone	50-22-6	95			100				Blair et al. (2000)
256	MUC	Cortisol	50-23-7				10				Korach (1979)
257	GST-aERdef	Coumestrol	479-13-0		0.1	0.04			3.1	0.491	Matthews et al. (2000)
258	GST-cERdef	Coumestrol	479-13-0		0.46	0.1			0.7	-0.155	Matthews et al. (2000)
259	GST-hER def	Coumestrol	479-13-0		0.036	0.03			0.81	-0.092	Matthews et al. (2000)
260	GST-mER def	Coumestrol	479-13-0		0.8	0.32			0.33	-0.481	Matthews et al. (2000)
261	GST-rERdef	Coumestrol	479-13-0		1.4	0.1			0.24	-0.620	Matthews et al. (2000)
262	hER	Coumestrol	479-13-0					0.00014	94	1.970	Kuiper et al. (1997)
263	hER	Coumestrol	479-13-0						20	1.301	Kuiper et al. (1998) [method a]
264	hER	Coumestrol	479-13-0		0.0027				34	1.532	Kuiper et al. (1998) [method b]
265	hER -FP	Coumestrol	479-13-0		0.109	0.001			12	1.079	Nikov et al. (2000)
266	hER	Coumestrol	479-13-0						140	2.146	Kuiper et al. (1998) [method a]
267	hER	Coumestrol	479-13-0		0.0011				100	2.000	Kuiper et al. (1998) [method b]
268	MCF-7 cytosol	Coumestrol	479-13-0		0.01				13	1.114	Dodge et al. (1996)
269	rER	Coumestrol	479-13-0					0.00007	185	2.267	Kuiper et al. (1997)
270	RUC	Coumestrol	479-13-0						0.9	-0.045	Fang et al. (2001)
271	RUC	Coumestrol	479-13-0					0.093	2.82	0.450	Waller et al. (1996)
272	RUC	p -Cumyl phenol	599-64-4						0.005	-2.301	Fang et al. (2001)
273	MCF-7 cells	Cyclofenil diphenol	5189-40-2						0.5	-0.301	Stoessel and Leclerq (1986)
274	MCF-7 cytosol	Cyclofenil diphenol	5189-40-2						5	0.699	Stoessel and Leclerq (1986)
275	MCF-7 cells	Cycloprop[14R ,15]estra-1,3,5(10)-triene 3,17 -diol, 3',15-dihydro-	73860-54-5						39	1.591	Stoessel and Leclerq (1986)
276	MCF-7 cytosol	Cycloprop[14R ,15]estra-1,3,5(10)-triene 3,17 -diol, 3',15-dihydro-	73860-54-5						45	1.653	Stoessel and Leclerq (1986)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
277	MCF-7 cells	Cycloprop[14S ,15b]estra-1,3,5(10)-triene-3,17 -diol, 3', 15-dihydro-	105455-76-3						81	1.908	Stoessel and Leclerq (1986)
278	MCF-7 cytosol	Cycloprop[14S ,15b]estra-1,3,5(10)-triene-3,17 -diol, 3', 15-dihydro-	105455-76-3						100	2.000	Stoessel and Leclerq (1986)
279	hER -FP	Cypermethrin	52315-07-8	> 93			10				Saito et al. (2000)
280	hER	Daidzein	486-66-8						0.1	-1.000	Kuiper et al. (1998) [method a]
281	hER	Daidzein	486-66-8		0.42				0.2	-0.699	Kuiper et al. (1998) [method b]
282	hER	Daidzein	486-66-8		23				0.022	-1.66	Morito et al. (2001)
283	hER -FP	Daidzein	486-66-8		7	1			0.2	-0.699	Nikov et al. (2000)
284	hER	Daidzein	486-66-8						0.5	-0.301	Kuiper et al. (1998) [method a]
285	hER	Daidzein	486-66-8		0.1				1	0.000	Kuiper et al. (1998) [method b]
286	hER	Daidzein	486-66-8		0.45				1.11	0.05	Morito et al. (2001)
287	RUC	Daidzein	486-66-8						0.023	-1.638	Fang et al. (2001)
288	RUC	<i>m,p</i> '-DDD	4329-12-8				100				Nelson (1974)
289	hER	<i>o,p</i> '-DDD	53-19-0				50				Klotz et al. (1996)
290	hER	<i>o,p</i> '-DDD	53-19-0				10				Kuiper et al. (1998) [method a]
291	hER	<i>o,p</i> '-DDD	53-19-0				10				Kuiper et al. (1998) [method a]
292	RUC	<i>o,p</i> '-DDD	53-19-0	99.2			300				Blair et al. (2000)
293	RUC	<i>o,p</i> '-DDD	53-19-0		10				0.009	-2.045	Nelson (1974)
294	hER	<i>p,p</i> '-DDD	72-54-8		11				0.009	-2.050	Klotz et al. (1996)
295	hER	<i>p,p</i> '-DDD	72-54-8				10				Kuiper et al. (1998) [method a]
296	hER	<i>p,p</i> '-DDD	72-54-8				10				Kuiper et al. (1998) [method a]
297	RUC	<i>p,p</i> '-DDD	72-54-8					1000	0.0003	-3.523	Waller et al. (1996)
298	RUC	<i>p,p</i> '-DDD	72-54-8	98.5			100				Blair et al. (2000)
299	RUC	<i>p,p</i> '-DDD	72-54-8				100				Nelson (1974)
300	hER	<i>o,p</i> '-DDE	3424-82-6				10				Kuiper et al. (1998) [method a]
301	hER	<i>o,p</i> '-DDE	3424-82-6				10				Kuiper et al. (1998) [method a]
302	GST-aERdef	<i>o,p</i> '-DDE	3424-82-6				100				Matthews et al. (2000)
303	GST-cERdef	<i>o,p</i> '-DDE	3424-82-6				100				Matthews et al. (2000)
304	GST-hER def	<i>o,p</i> '-DDE	3424-82-6				100				Matthews et al. (2000)
305	GST-mER def	<i>o,p</i> '-DDE	3424-82-6				100				Matthews et al. (2000)
306	GST-rtERdef	<i>o,p</i> '-DDE	3424-82-6		3.2	1			0.11	-0.959	Matthews et al. (2000)
307	RUC	<i>o,p</i> '-DDE	3242-82-6	99.8			500				Blair et al. (2000)
308	RUC	<i>o,p</i> '-DDE	3242-82-6		100				0.0009	-3.045	Nelson (1974)
309	hER	<i>p,p</i> '-DDE	72-55-9				10				Kuiper et al. (1998) [method a]
310	hER	<i>p,p</i> '-DDE	72-55-9				10				Kuiper et al. (1998) [method a]
311	RUC	<i>p,p</i> '-DDE	72-55-9				100				Nelson (1974)
312	RUC	<i>p,p</i> '-DDE	72-55-9					1000	0.0003	-3.523	Waller et al. (1996)
313	GST-aERdef	<i>p,p</i> '-DDE	72-55-9				100				Matthews et al. (2000)
314	GST-cERdef	<i>p,p</i> '-DDE	72-55-9				100				Matthews et al. (2000)
315	GST-hER def	<i>p,p</i> '-DDE	72-55-9				100				Matthews et al. (2000)
316	GST-mER def	<i>p,p</i> '-DDE	72-55-9				100				Matthews et al. (2000)
317	GST-rtERdef	<i>p,p</i> '-DDE	72-55-9		8	0.6			0.042	-1.377	Matthews et al. (2000)
318	RBC	<i>p,p</i> '-DDE	72-55-9	99.7			100				Andersen et al. (1999)
319	RUC	<i>p,p</i> '-DDE	72-55-9	99.4			100				Blair et al. (2000)
320	GST-rtERdef	<i>o,p</i> '-DDT	789-02-6		0.78	0.01			0.43	-0.367	Matthews et al. (2000)
321	hER	<i>o,p</i> '-DDT	789-02-6		1				0.1	-1.000	Klotz et al. (1996)
322	hER	<i>o,p</i> '-DDT	789-02-6						0.01	-2.000	Kuiper et al. (1998) [method a]
323	hER	<i>o,p</i> '-DDT	789-02-6						0.02	-1.699	Kuiper et al. (1998) [method a]

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
324	MUC	<i>o,p'</i> -DDT	789-02-6	> 99	0.875				0.210	-0.678	Shelby et al. (1996)
325	RBC	<i>o,p'</i> -DDT	789-02-6	99.8	3.4				0.00059	-3.229	Andersen et al. (1999)
326	RUC	<i>o,p'</i> -DDT	789-02-6		13.1			0.44	0.011	-1.959	McBlain (1987)
327	RUC	<i>o,p'</i> -DDT	789-02-6					2.89	0.09	-1.046	Waller et al. (1996)
328	hER -FP	<i>o,p'</i> -DDT	789-02-6	99	2.7				0.4	-0.398	Bolger et al. (1998)
329	GST-aERdef	<i>o,p'</i> -DDT	789-02-6				100				Matthews et al. (2000)
330	GST-cERdef	<i>o,p'</i> -DDT	789-02-6		3.7	1.2			0.086	-1.066	Matthews et al. (2000)
331	GST-hER def	<i>o,p'</i> -DDT	789-02-6				100				Matthews et al. (2000)
332	GST-mER def	<i>o,p'</i> -DDT	789-02-6		36	35			0.0073	-2.137	Matthews et al. (2000)
333	MCF-7 cytosol	<i>o,p'</i> -DDT	789-02-6		485	42			0.00031	-3.509	Soto et al. (1995)
334	MCF-7 cells	(-)- <i>o,p'</i> -DDT	58633-26-4		3				0.013	-1.88	Lascombe et al. (2000)
335	RUC	<i>o,p'</i> -DDT	789-02-6	98.5	64.3	8.9			0.001	-2.850	Blair et al. (2000)
336	RUC	<i>o,p'</i> -DDT	789-02-6		8.544			3.2	0.013	-1.900	Laws et al. (2000)
337	RUC	<i>o,p'</i> -DDT	789-02-6		2				0.045	-1.346	Nelson (1974)
338	RUC	(-)- <i>o,p'</i> -DDT	58633-26-4		5			0.17	0.029	-1.538	McBlain (1987)
339	MCF-7 cells	(+)- <i>o,p'</i> -DDT	58633-27-5		400				0.0001	-4.00	Lascombe et al. (2000)
340	RUC	(+)- <i>o,p'</i> -DDT	58633-27-5				20				McBlain (1987)
341	hER	<i>p,p'</i> -DDT	50-29-3				10				Kuiper et al. (1998) [method a]
342	hER -FP	<i>p,p'</i> -DDT	50-29-3	98	32				0.041	-1.387	Bolger et al. (1998)
343	hER	<i>p,p'</i> -DDT	50-29-3				10				Kuiper et al. (1998) [method a]
344	GST-aERdef	<i>p,p'</i> -DDT	50-29-3				100				Matthews et al. (2000)
345	GST-cERdef	<i>p,p'</i> -DDT	50-29-3				100				Matthews et al. (2000)
346	GST-hER def	<i>p,p'</i> -DDT	50-29-3				100			n.a	Matthews et al. (2000)
347	GST-mER def	<i>p,p'</i> -DDT	50-29-3				100				Matthews et al. (2000)
348	GST-rERdef	<i>p,p'</i> -DDT	50-29-3		2	0.4			0.165	-0.783	Matthews et al. (2000)
349	RUC	<i>p,p'</i> -DDT	50-29-3					1000	0.0003	-3.523	Waller et al. (1996)
350	RUC	<i>p,p'</i> -DDT	50-29-3	99.2			1000				Blair et al. (2000)
351	RUC	<i>p,p'</i> -DDT	50-29-3				100				Nelson (1974)
352	GST-aERdef	Dehydroepiandrosterone	53-43-0				100				Matthews et al. (2000)
353	GST-cERdef	Dehydroepiandrosterone	53-43-0				100				Matthews et al. (2000)
354	GST-hER def	Dehydroepiandrosterone	53-43-0				100				Matthews et al. (2000)
355	GST-mER def	Dehydroepiandrosterone	53-43-0				100				Matthews et al. (2000)
356	GST-rERdef	Dehydroepiandrosterone	53-43-0		12	2			0.028	-1.553	Matthews et al. (2000)
357	hER	Dehydroepiandrosterone	53-43-0					0.25	0.04	-1.398	Kuiper et al. (1997)
358	rER	Dehydroepiandrosterone	53-43-0					0.16	0.07	-1.155	Kuiper et al. (1997)
359	RUC	14-Dehydroestradiol-17	58699-19-7						107	2.029	Gabbard and Segaloff (1983)
360	MCF-7 cytosol	9, 11-Dehydroestradiol							196	2.292	Palomino et al. (1994)
361	RUC	14-Dehydroestradiol-17 3-methyl ether	35664-58-7						0.8	-0.097	Gabbard and Segaloff (1983)
362	RUC	14-Dehydroestrone	2119-18-8						9	0.954	Gabbard and Segaloff (1983)
363	RUC	14-Dehydroestrone 3-methyl ether	17550-11-7				1				Gabbard and Segaloff (1983)
364	MCF-7 cells	3-Deoxyestradiol	2529-64-8						0.6	-0.222	Brooks et al. (1987)
365	MCF-7 cytosol	3-Deoxyestradiol	2529-64-8						8	0.903	Brooks et al. (1987)
366	RUC	3-Deoxyestradiol	2529-64-8		0.18	0.02			0.50	-0.300	Blair et al. (2000)
367	RUC	3-Deoxyestrone	53-45-2		14.3	5.8			0.006	-2.200	Blair et al. (2000)
368	MUC	(<i>R</i>)-4'-Deoxyindenesol A	138515-00-1	> 98	0.556				0.20	-0.699	Chae et al. (1991)
369	MUC	(<i>rac</i>)-4'-Deoxyindenesol A		> 98	0.0756				1.30	0.114	Chae et al. (1991)
370	MUC	(<i>S</i>)-4'-Deoxyindenesol A	138514-99-5	> 98	0.0644				1.80	0.255	Chae et al. (1991)
371	MUC	(<i>R</i>)-5-Deoxyindenesol A	138515-02-3	> 98	0.117				0.90	-0.046	Chae et al. (1991)

Data Sorted by Substance and Assay

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1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
372	MUC	(rac)-5-Deoxyindenestrol A	138472-84-1	> 98	0.027				3.70	0.568	Chae et al. (1991)
373	MUC	(S)-5-Deoxyindenestrol A	138515-01-2	> 98	0.0177				5.60	0.748	Chae et al. (1991)
374	MCF-7 cells	17-Desoeyestradiol	53-63-4						0.5	-0.301	Brooks et al. (1987)
375	MCF-7 cytosol	17-Desoeyestradiol	53-63-4						40	1.602	Brooks et al. (1987)
376	RUC	17-Desoeyestradiol	53-63-4		0.00885	0.0032			10.16	1.010	Blair et al. (2000)
377	RUC	17-Desoeyestradiol	53-63-4		0.00495	0.00085			18.16	1.260	Blair et al. (2000)
378	RUC	17-Desoeyestradiol	53-63-4		0.009				55.5	1.744	Elsby et al. (2000)
379	RUC	Dexamethasone	50-02-2				100				Blair et al. (2000)
380	RUC	1,3-Diacetoxy-17-ethynyl-7-methyl-1,3,5(10)-estratrien-17-ol			0.008				20	1.301	Leibl and Spona (1982)
381	RUC	4,4'-Diaminostilbene dihydrochloride	66635-40-3	95			100				Blair et al. (2000)
382	MCF-7 cells	Dibenz[ah]anthracene	53-70-3				5				Arcaro et al. (1999)
383	RUC	Dibenzo-18-crown-6	14187-32-7	98			10				Blair et al. (2000)
384	RUC	1,3-Dibenzoyloxy-17-ethynyl-7-methyl-1,3,5(10)-estratrien-17-ol			0.022				7.3	0.863	Leibl and Spona (1982)
385	RUC	1,3-Dibenzyltetramethyldisiloxane					100				Fang et al. (2001)
386	GST-aERdef	Dibutyl benzyl phthalate					100				Matthews et al. (2000)
387	GST-cERdef	Dibutyl benzyl phthalate					100				Matthews et al. (2000)
388	GST-hER def	Dibutyl benzyl phthalate					100				Matthews et al. (2000)
389	GST-mER def	Dibutyl benzyl phthalate					100				Matthews et al. (2000)
390	GST-rERdef	Dibutyl benzyl phthalate			1.7	2.3			0.2	-0.699	Matthews et al. (2000)
391	RUC	2,6-Di-tert-butylphenol	128-39-2	98			100				Blair et al. (2000)
392	hER -FP	Dibutyl phthalate	84-74-2	> 98			5000				Hashimoto et al. (2000)
393	RBC	Dibutyl phthalate	84-74-2	98			100				Andersen et al. (1999)
394	RUC	Dibutyl phthalate	84-74-2	99			1000				Blair et al. (2000)
395	RUC	Dibutyl phthalate	84-74-2					100.46	0.0026	-2.590	Waller et al. (1996)
396	RUC	Dibutyl phthalate	84-74-2	99.9	47				0.0028	-2.553	Zacharewski et al. (1998)
397	RUC	2,4'-Dichlorobiphenyl	34883-43-7	99	365	115			0.0002	-3.610	Blair et al. (2000)
398	hER	2,5-Dichlorobiphenyl	34883-39-1	≥ 99%			50				Vakharia and Gierthy (2000)
399	MUC	2',6'-Dichloro-4-biphenylol	79881-33-7	> 98	0.3880				0.26	-0.588	Korach et al. (1988)
400	hER	3,4-Dichlorobiphenyl	2974-92-7	≥ 99%			50				Vakharia and Gierthy (2000)
401	hER	3,5-Dichlorobiphenyl	34883-41-5	≥ 99%			50				Vakharia and Gierthy (2000)
402	RUC	4,4'-Dichlorobiphenyl	2050-68-2	98.6			300				Blair et al. (2000)
403	hER	2,5-Dichloro-2'-biphenylol	53905-30-9	≥ 99%			50				Vakharia and Gierthy (2000)
404	hER	2,5-Dichloro-3'-biphenylol	53905-29-6	≥ 99%	50				0.002	-2.700	Vakharia and Gierthy (2000)
405	hER	2',5'-Dichloro-4-biphenylol	53905-28-5	≥ 99%	3				0.033	-1.480	Vakharia and Gierthy (2000)
406	MUC	2',5'-Dichloro-4-biphenylol	53905-28-5	> 98	0.5060				0.198	-0.703	Korach et al. (1988)
407	MUC	2',5'-Dichloro-4-biphenylol	53905-28-5					0.36	0.72	-0.140	Waller et al. (1996)
408	RUC	2',5'-Dichloro-4-biphenylol	53905-28-5	95	2.5	0.3			0.036	-1.440	Blair et al. (2000)
409	hER	3,4-Dichloro-2'-biphenylol	209613-97-8	95 - 99			50				Vakharia and Gierthy (2000)
410	hER	3,4-dichloro-3'-biphenylol	14962-34-6	95 - 99			50				Vakharia and Gierthy (2000)
411	hER	3,4-dichloro-4'-biphenylol	53890-77-0	95 - 99	0.33				0.30	-0.519	Vakharia and Gierthy (2000)
412	hER	3,5-Dichloro-2'-biphenylol		≥ 99%			50				Vakharia and Gierthy (2000)
413	hER	3,5-Dichloro-4'-biphenylol		≥ 99%			50				Vakharia and Gierthy (2000)
414	RUC	3,5-Dichloro-2-hydroxy-2-methylbut-3-enalide	16776-82-1	> 99			200				Laws et al. (1996)
415	RUC	3,5-Dichloro-2-hydroxy-2-methylbut-3-enalide	16776-82-1					1000	0.0003	-3.523	Waller et al. (1996)
416	RUC	2,4-Dichlorophenoxyacetic acid	94-75-7	99			100				Blair et al. (2000)
417	RUC	2-[[[(3,5-Dichlorophenyl)amino]-carbamoyl]oxy]-2-methyl-3-butenic acid	119209-27-7					1000	0.0003	-3.523	Waller et al. (1996)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
418	RUC	2-[[[(3,5-Dichlorophenyl)amino]-carbonyl]oxy]-2-methyl-3-butenic acid	119209-27-7				500				Laws et al. (1996)
419	hER	Dieldrin	60-57-1	95 - 99			10				Arcaro et al. (2000)
420	hER -FP	Dieldrin	60-57-1	98.8	2485				0.0005	-3.301	Bolger et al. (1998)
421	MUC	Dieldrin	60-57-1	Technical grade			10				Ramamoorthy et al. (1997a)
422	RUC	Dieldrin	60-57-1	98			100				Blair et al. (2000)
423	RUC	Dieldrin	60-57-1	90			100				Blair et al. (2000)
424	hER	Dienestrol	84-17-3					0.00005	223	2.348	Kuiper et al. (1997)
425	rER	Dienestrol	84-17-3					0.00003	404	2.606	Kuiper et al. (1997)
426	RUC	Dienestrol	84-17-3	99	0.0024	0			37.46	1.570	Blair et al. (2000)
427	MUC	-Dienestrol	13029-44-2		0.005	0.0008			32	1.500	Korach et al. (1978)
428	MUC	-Dienestrol	35495-11-5		0.367	0.072			0.44	-0.357	Korach et al. (1978)
429	RUC	1,3-Diethyl-6,4'-dihydroxy-2-phenylindene							79	1.898	Anstead et al. (1989)
430	hER -FP	Di-2-ethylhexyl adipate	103-23-1	99			5000				Hashimoto et al. (2000)
431	hER -FP	Diethylhexyl phthalate	117-81-7	99			5000				Hashimoto et al. (2000)
432	RUC	Diethylhexyl phthalate	117-81-7	99			1000				Blair et al. (2000)
433	RUC	Diethylhexyl phthalate	117-81-7	99.9			1000				Zacharewski et al. (1998)
434	RUC	1,3-Diethyl-4-hydroxy-2-phenylindene							9.3	0.968	Anstead et al. (1989)
435	RUC	1,3-Diethyl-6-hydroxy 2-phenylindene							2.2	0.342	Anstead et al. (1989)
436	RUC	<i>meso-p</i> -(, -Diethyl- <i>p</i> -methylphenethyl)phenol	267408-76-4		0.0225	0.0075			4.00	0.600	Blair et al. (2000)
437	RUC	Diethyl phthalate	84-66-2	99			1000				Blair et al. (2000)
438	GST-aERdef	Diethylstilbestrol	56-53-1		0.0029	0.0001			107	2.029	Matthews et al. (2000)
439	GST-cERdef	Diethylstilbestrol	56-53-1		0.0025	0.0006			130	2.114	Matthews et al. (2000)
440	GST-hER def	Diethylstilbestrol	56-53-1		0.0032	0.0001			91	1.959	Matthews et al. (2000)
441	GST-mER def	Diethylstilbestrol	56-53-1		0.0032	0.0005			84	1.924	Matthews et al. (2000)
442	GST-rERdef	Diethylstilbestrol	56-53-1		0.002	0.0001			165	2.217	Matthews et al. (2000)
443	hER	Diethylstilbestrol	56-53-1					0.00004	468	2.670	Kuiper et al. (1997)
444	hER	Diethylstilbestrol	56-53-1						236	2.373	Kuiper et al. (1998) [method a]
445	hER	Diethylstilbestrol	56-53-1		0.0075				66.7	1.82	Morito et al. (2001)
446	hER -FP	Diethylstilbestrol	56-53-1	99	0.011				118	2.072	Bolger et al. (1998)
447	hER -FP	Diethylstilbestrol	56-53-1		0.01	0.0005			130	2.114	Nikov et al. (2001)
448	hER -FP	Diethylstilbestrol	56-53-1		0.0035				160	2.204	Parker et al. (2000)
449	hER -FP	Diethylstilbestrol	56-53-1	> 93	0.07				57	1.756	Saito et al. (2000)
450	hER	Diethylstilbestrol	56-53-1						221	2.344	Kuiper et al. (1998) [method a]
451	hER	Diethylstilbestrol	56-53-1		0.005				100	2.00	Morito et al. (2001)
452	MCF-7 cells	Diethylstilbestrol	56-53-1						84	1.924	Stoessel and Leclerq (1986)
453	MCF-7 cytosol	Diethylstilbestrol	56-53-1					0.00018	100	2.000	Rijks et al. (1996)
454	MCF-7 cytosol	Diethylstilbestrol	56-53-1						100	2.000	Stoessel and Leclerq (1986)
455	MUC	Diethylstilbestrol	56-53-1						90	1.950	Korach (1979)
456	MUC	Diethylstilbestrol	56-53-1		0.002	0.0003			80	1.900	Korach et al. (1978)
457	MUC	Diethylstilbestrol	56-53-1		0.001	0.0001			100	2.000	Korach et al. (1979)
458	MUC	Diethylstilbestrol	56-53-1		0.5	0.1			320	2.510	Korach et al. (1985)
459	MUC	Diethylstilbestrol	56-53-1		0.0004				250	2.398	Korach et al. (1988)
460	MUC	Diethylstilbestrol	56-53-1						286	2.460	Korach et al. (1989)
461	MUC	Diethylstilbestrol	56-53-1	> 99	0.0016				113	2.050	Shelby et al. (1996)
462	RBC	Diethylstilbestrol	56-53-1	99	7				2857	3.456	Andersen et al. (1999)
463	RBC	Diethylstilbestrol	56-53-1						246	2.391	Korenman (1969)
464	rER	Diethylstilbestrol	56-53-1					0.00004	295	2.470	Kuiper et al. (1997)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)††	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
465	RUC	Diethylstilbestrol	56-53-1		100				0.003	-2.523	Ashby et al. (1999)
466	RUC	Diethylstilbestrol	56-53-1		0.000225	0.000005			399.56	2.600	Blair et al. (2000)
467	RUC	Diethylstilbestrol	56-53-1		0.0001				5000	3.699	Elsby et al. (2000)
468	RUC	Diethylstilbestrol	56-53-1		0.0014				214	2.330	Laws et al. (1996)
469	RUC	Diethylstilbestrol	56-53-1		0.0005			0.0002	200	2.300	Laws et al. (2000)
470	RUC	Diethylstilbestrol	56-53-1		0.00019				100	2.000	Liu et al. (1994)
471	RUC	Diethylstilbestrol	56-53-1		0.00031			0.00001	471	2.673	McBlain (1987)
472	RUC	Diethylstilbestrol	56-53-1		0.001				90	1.950	Nelson (1974)
473	RUC	Diethylstilbestrol	56-53-1					0.00016	100	2.000	Rijks et al. (1996)
474	RUC	Diethylstilbestrol	56-53-1	> 99	0.0002				100	2.000	Routledge et al. (1998)
475	RUC	Diethylstilbestrol	56-53-1					0.0006998	371	2.569	Waller et al. (1996)
476	MCF-7 cells	3,3'-Diethylstilbestrol	5959-71-7						17.5	1.243	Stoessel and Leclercq (1986)
477	MCF-7 cytosol	3,3'-Diethylstilbestrol	5959-71-7						3	0.477	Stoessel and Leclercq (1986)
478	RUC	Diethylstilbestrol dimethyl ether	130-79-0		1.6	0.3			0.056	-1.250	Blair et al. (2000)
479	MUC	Diethylstilbestrol epoxide	6052-82-0		0.017	0.002			9.4	0.970	Korach et al. (1978)
480	MUC	Diethylstilbestrol-phenanthrene			0.6	0.173			0.27	-0.569	Korach et al. (1978)
481	hER	(rac) 5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							14	1.146	Meyers et al. (1999)
482	hER	(rac) 5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							67	1.830	Meyers et al. (1999)
483	hER	(5R ,11R)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							23	1.361	Meyers et al. (1999)
484	hER	(5R ,11R)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							144	2.160	Meyers et al. (1999)
485	hER	(5S ,11S)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							14	1.150	Meyers et al. (1999)
486	hER	(5S ,11S)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							0.9	-0.046	Meyers et al. (1999)
487	hER	(trans)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							221	2.344	Meyers et al. (1999)
488	hER	(trans)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							432	2.640	Meyers et al. (1999)
489	RUC	Dihexyl phthalate	84-75-3	99.6			1000				Zacharewski et al. (1998)
490	RUC	5,6-Dihydro-8-[2-(dimethylamino)ethoxy]-12-ethyl-11-phenyl-dibenzo[a,e]-cyclooctene, hydrate (1:4)	85850-78-8						0.22	-0.658	Acton et al. (1983)
491	hER	Dihydrogenistein	21554-71-2		3.5				0.143	-0.84	Morito et al. (2001)
492	hER	Dihydrogenistein	21554-71-2		0.027				18.5	1.27	Morito et al. (2001)
493	hER	Dihydroglycitein	94105-88-1		22				0.023	-1.64	Morito et al. (2001)
494	GST-aERdef	5 -Dihydrotestosterone	521-18-6		0.82	0.12			0.38	-0.420	Matthews et al. (2000)
495	GST-cERdef	5 -Dihydrotestosterone	521-18-6		38	6			0.0085	-2.071	Matthews et al. (2000)
496	GST-hER def	5 -Dihydrotestosterone	521-18-6		5.9	0.9			0.049	-1.310	Matthews et al. (2000)
497	GST-mER def	5 -Dihydrotestosterone	521-18-6		6.6	1.4			0.04	-1.398	Matthews et al. (2000)
498	GST-rtERdef	5 -Dihydrotestosterone	521-18-6		10	3			0.034	-1.469	Matthews et al. (2000)
499	hER	5 -Dihydrotestosterone	521-18-6					0.22	0.05	-1.300	Kuiper et al. (1997)
500	hER -FP	5 -Dihydrotestosterone	521-18-6	99	136				0.0095	-2.022	Bolger et al. (1998)
501	rER	5 -Dihydrotestosterone	521-18-6					0.073	0.17	-0.770	Kuiper et al. (1997)
502	RUC	5 -Dihydrotestosterone	521-18-6					10	0.026	-1.590	Waller et al. (1996)
503	MUC	5 -Dihydrotestosterone	521-18-6				10				Korach (1979)
504	RUC	5 -Dihydrotestosterone	521-18-6	99					0.001	-3.000	Fang et al. (2001)
505	RUC	5 -Dihydrotestosterone	571-22-2	99			100				Blair et al. (2000)
506	RUC	2,2'-Dihydroxybenzophenone	835-11-0	98			100				Blair et al. (2000)
507	RUC	2,4-Dihydroxybenzophenone	131-56-6	99	36.5	4.5			0.002	-2.610	Blair et al. (2000)

Data Sorted by Substance and Assay

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1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
508	RUC	4,4'-Dihydroxybenzophenone	611-99-4	99	26	4			0.003	-2.460	Blair et al. (2000)
509	RUC	4,4'-Dihydroxybenzophenone	611-99-4	97					0.013	-1.886	Perez et al. (1998)
510	hER	4,4'-Dihydroxybiphenyl	92-88-6				10				Kuiper et al. (1998) [method a]
511	hER	4,4'-Dihydroxybiphenyl	92-88-6						0.03	-1.523	Kuiper et al. (1998) [method a]
512	MUC	4,4'-Dihydroxybiphenyl	92-88-6	> 98			5				Korach et al. (1988)
513	MUC	Dihydroxydiethylstilbestrol	7507-01-9		0.334	0.092			0.48	-0.319	Korach et al. (1978)
514	RUC	6,4'-Dihydroxyflavone	63046-09-3						0.15	-0.820	Fang et al. (2001)
515	RUC	3,3'-Dihydroxyhexestrol	79199-51-2		0.00585	0.00165			15.37	1.190	Blair et al. (2000)
516	RUC	2,2'-Dihydroxy-4-methoxybenzophenone	131-53-3	98			100				Blair et al. (2000)
517	RUC	3-(2,3 Dihydroxypropoxy)-10-ethyl-11-phenyldibenz- <i>[b,f]</i> joxepin	85850-89-1						0.07	-1.155	Acton et al. (1983)
518	RUC	Diisobutyl phthalate	84-69-5	98			1000				Blair et al. (2000)
519	RUC	Diisodecyl phthalate	26761-40-0	99.6			1000				Zacharewski et al. (1998)
520	RUC	Diisoheptyl phthalate	41451-28-9	99.6			1000				Zacharewski et al. (1998)
521	RUC	Diisononyl phthalate	28553-12-0	Technical grade			1000				Blair et al. (2000)
522	RUC	Diisononyl phthalate	28553-12-0	99.8			1000				Zacharewski et al. (1998)
523	RUC	11-[2-(<i>N,N'</i> -Dimethylamino)-ethoxy]estra-1,3,5 (10)-triene-3,17b-diol							1.6	0.204	Qian and Abul-Hajj (1990)
524	RUC	3-[2-(Dimethylamino)ethoxy]-11-ethyl-12-phenyl-6 <i>H</i> -dibenzo[<i>b,f</i>]thiactin	85850-79-9						1.1	0.041	Acton et al. (1983)
525	RUC	3-[2-(Dimethylamino)ethoxy]-10-ethyl-11-phenyldibenz- <i>[b,f]</i> oxepin	85850-76-6						0.02	-1.699	Acton et al. (1983)
526	RUC	7-[2-(Dimethylamino)ethoxy]-11-ethyl-10-phenyldibenz- <i>[b,f]</i> thiepin	85850-77-7						0.12	-0.921	Acton et al. (1983)
527	RUC	11-[3-(<i>N,N'</i> -Dimethylamino)-propoxy]estra-1,3,5 (10)-triene-3,17-diol	130043-38-8						2.6	0.415	Qian and Abul-Hajj (1990)
528	RUC	, -Dimethyl- -ethylallenolic acid	15372-37-9		0.095	0.005			0.95	-0.020	Blair et al. (2000)
529	RUC	2,6-Dimethylhexestrol	334707-28-7		0.007	0.00173			12.84	1.110	Blair et al. (2000)
530	RUC	1,6-Dimethylnaphthalene	575-43-9	99			100				Blair et al. (2000)
531	RUC	Dimethyl phthalate	131-11-3	99			1000				Blair et al. (2000)
532	RBC	, -Dimethylstilbestrol	552-80-7						129	2.111	Korenman (1970)
533	RUC	, -Dimethylstilbestrol	552-80-7		0.0062	0.0013			14.50	1.160	Blair et al. (2000)
534	MCF-7 cells	Dimethyl sulfoxide	67-68-5				5				Arcaio et al. (1999)
535	hER	5,11- <i>trans</i> -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							222	2.346	Meyers et al. (1999)
536	hER	5,11- <i>trans</i> -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							254	2.400	Meyers et al. (1999)
537	hER	(<i>SR</i> ,11 <i>R</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							24	1.380	Meyers et al. (1999)
538	hER	(<i>SR</i> ,11 <i>R</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							76	1.880	Meyers et al. (1999)
539	hER	(<i>SS</i> ,11 <i>S</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							9.3	0.968	Meyers et al. (1999)
540	hER	(<i>SS</i> ,11 <i>S</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							75	1.880	Meyers et al. (1999)
541	RUC	Di- <i>n</i> -octyl phthalate	117-84-0	98			1000				Blair et al. (2000)
542	RUC	Di- <i>n</i> -octyl phthalate	117-84-0	98.7			1,000				Zacharewski et al. (1998)
543	RUC	Diphenolic acid	126-00-1	95	120	30			0.0007	-3.130	Blair et al. (2000)
544	RUC	<i>trans, trans</i> -1,4-Diphenyl-1,3-butadiene	886-65-7	98			100				Blair et al. (2000)
545	RUC	4-[1,2-(Diphenyl-1-butenyl)]phenol acetate	100808-55-7						21	1.322	Jordan et al. (1986)
546	RUC	2,3-Diphenylindene-1							0.0095	-2.022	Anstead et al. (1989)
547	RUC	4-[1-(Diphenylmethylene)-propyl]phenol acetate	82333-68-4						2	0.301	Jordan et al. (1986)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)††	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
548	RUC	1,3-Diphenyltetramethyldisiloxane	56-33-7						0.0007	-3.155	Fang et al. (2001)
549	hER	5,11- <i>trans</i> -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							33.6	1.526	Meyers et al. (1999)
550	hER	5,11- <i>trans</i> -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							92.3	1.970	Meyers et al. (1999)
551	hER	(5 <i>R</i> ,11 <i>R</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							5.2	0.716	Meyers et al. (1999)
552	hER	(5 <i>R</i> ,11 <i>R</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							26	1.410	Meyers et al. (1999)
553	hER	(5 <i>S</i> ,11 <i>S</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1.6	0.204	Meyers et al. (1999)
554	hER	(5 <i>S</i> ,11 <i>S</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							5.1	0.710	Meyers et al. (1999)
555	RUC	4-Dodecylphenol	104-43-8	99.7	4.85	1.95			0.019	-1.730	Blair et al. (2000)
556	RUC	Doisynoestrol	15372-34-6		49	14			0.002	-2.740	Blair et al. (2000)
557	RUC	Dopamine	51-61-6	99			100				Blair et al. (2000)
558	RUC	Droloxifene	82413-20-5		0.0059	0.0031			15.24	1.180	Blair et al. (2000)
559	MCF-7 cells	Droloxifene	82413-20-5						0.2	-0.699	Stoessel and Leclercq (1986)
560	MCF-7 cytosol	Droloxifene	82413-20-5						2.5	0.398	Stoessel and Leclercq (1986)
561	hER -FP	Empenthrin	54406-48-3	> 93			10				Saito et al. (2000)
562	hER	-Endosulfan	959-98-8	95 - 99			10				Arcaro et al. (2000)
563	GST-aERdef	-Endosulfan	959-98-8				100				Matthews et al. (2000)
564	GST-cERdef	-Endosulfan	959-98-8				100				Matthews et al. (2000)
565	GST-hER def	-Endosulfan	959-98-8				100				Matthews et al. (2000)
566	GST-mER def	-Endosulfan	959-98-8				100				Matthews et al. (2000)
567	GST-rtERdef	-Endosulfan	959-98-8		28	14			0.012	-1.921	Matthews et al. (2000)
568	hER	, -Endosulfan	115-29-7				10				Kuiper et al. (1998) [method a]
569	hER	, -Endosulfan	115-29-7				10				Kuiper et al. (1998) [method a]
570	MUC	, -Endosulfan	115-29-7	98 (a=78%, b=20%)			5				Shelby et al. (1996)
571	RBC	, -Endosulfan	115-29-7	99			100				Andersen et al. (1999)
572	RUC	, -Endosulfan	115-29-7	99			1000				Blair et al. (2000)
573	RUC	, -Endosulfan	115-29-7					599.79	0.00044	-3.360	Waller et al. (1996)
574	GST-mER def	-Endosulfan	33213-65-9				100				Matthews et al. (2000)
575	hER	-Endosulfan	33213-65-9	95 - 99			10				Arcaro et al. (2000)
576	GST-aERdef	-Endosulfan	33213-65-9				100				Matthews et al. (2000)
577	GST-cERdef	-Endosulfan	33213-65-9				100				Matthews et al. (2000)
578	GST-hER def	-Endosulfan	33213-65-9				100				Matthews et al. (2000)
579	GST-rtERdef	-Endosulfan	33213-65-9				100				Matthews et al. (2000)
580	MCF-7 cytosol	-Endosulfan	33213-65-9	Technical grade	631	88			0.00024	-3.620	Soto et al. (1995)
581	RBC	16-Epiestriol	547-81-9						44	1.643	Korenman (1969)
582	hER	17-Epiestriol	1228-72-4						29	1.462	Kuiper et al. (1998) [method a]
583	hER	17-Epiestriol	1228-72-4						80	1.903	Kuiper et al. (1998) [method a]
584	RUC	Epitestosterone	481-30-1	99.9			600				Blair et al. (2000)
585	RBC	Equilenin	517-09-9						8	0.903	Korenman (1969)
586	RBC	Equilin	474-86-2						24	1.380	Korenman (1969)
587	hER	Equol	531-95-3		1.5				0.33	-0.48	Morito et al. (2001)
588	hER	Equol	531-95-3		0.0085				58.8	1.77	Morito et al. (2001)
589	RUC	Equol	531-95-3						0.15	-0.820	Fang et al. (2001)
590	RBC	<i>erythro</i> -MEA	20576-52-7						135	2.130	Korenman (1970)
591	RBC	16 -Estradiol	1090-04-6						66	1.820	Korenman (1969)
592	MCF-7 cytosol	16 -Estradiol	1090-04-6						35	1.544	Brooks et al. (1987)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
593	MCF-7 cytosol	16 -Estradiol	1090-04-6						0.8	-0.097	VanderKuur et al. (1993)
594	hER	17 -Estradiol	57-91-0					0.0002	58	1.760	Kuiper et al. (1997)
595	hER	17 -Estradiol	57-91-0						7	0.845	Kuiper et al. (1998) [method a]
596	hER	17 -Estradiol	57-91-0						2	0.301	Kuiper et al. (1998) [method a]
597	MCF-7 cytosol	17 -Estradiol	57-91-0						0.22	-0.658	VanderKuur et al. (1993)
598	MUC	17 -Estradiol	57-91-0						1000	3.000	Korach (1979)
599	RBC	17 -Estradiol	57-91-0						49	1.690	Korenman (1969)
600	rER	17 -Estradiol	57-91-0					0.0012	11	1.041	Kuiper et al. (1997)
601	RUC	17 -Estradiol	57-91-0	99	0.0293	0.008			3.07	0.490	Blair et al. (2000)
602	RUC	17 -Estradiol	57-91-0		0.01				50	1.699	Elsby et al. (2000)
603	GST-hER def	17 -Estradiol	50-28-2		0.0055	0.0012			100	2.00	Fertuck et al. (2001)
604	hER	17 -Estradiol	50-28-2		0.0006				100	2.000	Arcaro et al. (1999)
605	hER	17 -Estradiol	50-28-2		0.0005				100	2.000	Arcaro et al. (2000)
606	hER	17 -Estradiol	50-28-2	> 97	0.004				100	2.000	Gaido et al. (1999)
607	hER	17 -Estradiol	50-28-2		0.001				100.0	2.000	Klotz et al. (1996)
608	hER	17 -Estradiol	50-28-2						100	2.000	Kraichely et al. (2000)
609	hER	17 -Estradiol	50-28-2		0.00021			0.00013	100	2.000	Kuiper et al. (1997)
610	hER	17 -Estradiol	50-28-2						100	2.000	Kuiper et al. (1998) [method a]
611	hER	17 -Estradiol	50-28-2		0.00093				100	2.000	Kuiper et al. (1998) [method b]
612	hER	17 -Estradiol	50-28-2						100	2.000	Meyers et al. (1999)
613	hER	17 -Estradiol	50-28-2		0.005				100	2.00	Morito et al. (2001)
614	hER	17 -Estradiol	50-28-2					0.0003	100	2.000	Sun et al. (1999)
615	hER	17 -Estradiol	50-28-2		0.00059				100	2.000	Vakharia and Gierthy (1999)
616	hER	17 -Estradiol	50-28-2		0.001				100	2.000	Vakharia and Gierthy (2000)
617	hER -FP	17 -Estradiol	50-28-2	98	0.013				100	2.000	Bolger et al. (1998)
618	hER -FP	17 -Estradiol	50-28-2		0.0195	0.0018			100	2.000	Hanioka et al. (1999)
619	hER -FP	17 -Estradiol	50-28-2		0.01				100	2.000	Hashimoto et al. (2000)
620	hER -FP	17 -Estradiol	50-28-2		0.013	0.0007			100	2.000	Nikov et al. (2000)
621	hER -FP	17 -Estradiol	50-28-2		0.013	0.0007			100	2.000	Nikov et al. (2001)
622	hER -FP	17 -Estradiol	50-28-2		0.0056				100	2.000	Parker et al. (2000)
623	hER -FP	17 -Estradiol	50-28-2		0.04				100	2.000	Saito et al. (2000)
624	hER	17 -Estradiol	50-28-2		0.005				100	2.000	Arcaro et al. (1999)
625	hER	17 -Estradiol	50-28-2		0.0056	0.0011			100	2.00	Fertuck et al. (2001)
626	hER	17 -Estradiol	50-28-2	> 97					100	2.000	Gaido et al. (1999)
627	hER	17 -Estradiol	50-28-2						100	2.000	Kraichely et al. (2000)
628	hER	17 -Estradiol	50-28-2						100	2.000	Kuiper et al. (1998) [method a]
629	hER	17 -Estradiol	50-28-2						100	2.000	Kuiper et al. (1998) [method a]
630	hER	17 -Estradiol	50-28-2		0.00106				100	2.000	Kuiper et al. (1998) [method b]
631	hER	17 -Estradiol	50-28-2						100	2.000	Meyers et al. (1999)
632	hER	17 -Estradiol	50-28-2		0.005				100	2.00	Morito et al. (2001)
633	hER	17 -Estradiol	50-28-2					0.0009	100	2.000	Sun et al. (1999)
634	MCF-7 cells	17 -Estradiol	50-28-2						100	2.000	Arcaro et al. (1999)
635	MCF-7 cells	17 -Estradiol	50-28-2						100	2.000	Brooks et al. (1987)
636	MCF-7 cells	17 -Estradiol	50-28-2		0.0004				100	2.000	Lascombe et al. (2000)
637	MCF-7 cells	17 -Estradiol	50-28-2						100	2.000	Nagel et al. (1997)
638	MCF-7 cytosol	17 -Estradiol	50-28-2						100	2.000	Brooks et al. (1987)
639	MCF-7 cytosol	17 -Estradiol	50-28-2		0.0009				100	2.000	Dodge et al. (1996)
640	MCF-7 cytosol	17 -Estradiol	50-28-2		1.3	0.8			100	2.000	Kramer et al. (1997)
641	MCF-7 cytosol	17 -Estradiol	50-28-2						100	2.000	Palomino et al. (1994)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
642	MCF-7 cytosol	17 -Estradiol	50-28-2		0.0015	0.0004			100	2.000	Soto et al. (1995)
643	MCF-7 cytosol	17 -Estradiol	50-28-2						100	2.000	VanderKuur et al. (1993)
644	MUC	17 -Estradiol	50-28-2		0.0021				100	2.000	Connor et al. (1997)
645	MUC	17 -Estradiol	50-28-2		0.015				100	2.000	Fielden et al. (1997)
646	MUC	17 -Estradiol	50-28-2		0.001				100	2.000	Korach (1979)
647	MUC	17 -Estradiol	50-28-2		0.0016	0.0005			100	2.000	Korach et al. (1978)
648	MUC	17 -Estradiol	50-28-2		1.6	0.5			100	2.000	Korach et al. (1985)
649	MUC	17 -Estradiol	50-28-2		0.0010				100	2.000	Korach et al. (1988)
650	MUC	17 -Estradiol	50-28-2						100	2.000	Korach et al. (1989)
651	MUC	17 -Estradiol	50-28-2		0.003	0.0002			100	2.000	Matthews et al. (2001)
652	MUC	17 -Estradiol	50-28-2		0.0032				100	2.000	Ramamoorthy et al. (1997a)
653	MUC	17 -Estradiol	50-28-2		0.011				100	2.000	Ramamoorthy et al. (1997b)
654	MUC	17 -Estradiol	50-28-2	> 99	0.0018				100	2.000	Shelby et al. (1996)
655	RBC	17 -Estradiol	50-28-2						100	2.000	Korenman (1969)
656	rER	17 -Estradiol	50-28-2		0.00013			0.00012	100	2.000	Kuiper et al. (1997)
657	RUC	17 -Estradiol	50-28-2						100	2.000	Acton et al. (1983)
658	RUC	17 -Estradiol	50-28-2		0.003				100	2.000	Ashby et al. (1999)
659	RUC	17 -Estradiol	50-28-2						100	2.000	Gabbard and Segaloff (1983)
660	RUC	17 -Estradiol	50-28-2		0.008				100	2.000	Jaimez et al. (2000)
661	RUC	17 -Estradiol	50-28-2						100	2.000	Jordan et al. (1986)
662	RUC	17 -Estradiol	50-28-2		0.0011			0.0004	100	2.000	Laws et al. (2000)
663	RUC	17 -Estradiol	50-28-2		0.001				100	2.000	Olea et al. (1996)
664	RUC	17 -Estradiol	50-28-2						100	2.000	Qian and Abul-Hajj (1990)
665	RUC	17 -Estradiol	50-28-2					0.0026	100	2.000	Waller et al. (1996)
666	GST-aERdef	17 -Estradiol	50-28-2		0.0025	0.0013			100	2.000	Matthews and Zacharewski (2000)
667	GST-aERdef	17 -Estradiol	50-28-2		0.0031	0.0005			100	2.000	Matthews et al. (2000)
668	GST-cERdef	17 -Estradiol	50-28-2		0.0032	0.0005			100	2.000	Matthews et al. (2000)
669	GST-hER def	17 -Estradiol	50-28-2		0.0024	0.001			100	2.000	Matthews and Zacharewski (2000)
670	GST-hER def	17 -Estradiol	50-28-2		0.0029	0.0005			100	2.000	Matthews et al. (2000)
671	GST-mER def	17 -Estradiol	50-28-2		0.0027	0.0004			100	2.000	Matthews et al. (2000)
672	GST-rERdef	17 -Estradiol	50-28-2		0.0031	0.0006			100	2.000	Matthews and Zacharewski (2000)
673	GST-rERdef	17 -Estradiol	50-28-2		0.0033	0.0005			100	2.000	Matthews et al. (2000)
674	RBC	17 -Estradiol	50-28-2	99.4	0.00002				100	2.000	Andersen et al. (1999)
675	RUC	17 -Estradiol	50-28-2		0.00625				100	2.000	Allen et al. (1980)
676	RUC	17 -Estradiol	50-28-2		0.000899	0.000027			100	2.000	Blair et al. (2000)
677	RUC	17 -Estradiol	50-28-2		0.014				100	2.000	Connor et al. (1997)
678	RUC	17 -Estradiol	50-28-2		0.005				100	2.000	Elsby et al. (2000)
679	RUC	17 -Estradiol	50-28-2	98	0.003				100	2.000	Laws et al. (1996)
680	RUC	17 -Estradiol	50-28-2		0.0016				100	2.000	Leibl and Spona (1982)
681	RUC	17 -Estradiol	50-28-2		0.00146			0.00005	100	2.000	McBlain (1987)
682	RUC	17 -Estradiol	50-28-2		0.0009				100	2.000	Nelson (1974)
683	RUC	17 -Estradiol	50-28-2						100	2.000	Perez et al. (1998)
684	RUC	17 -Estradiol	50-28-2		0.0013				100	2.000	Zacharewski et al. (1998)
685	MCF-7 cytosol	9 -Estradiol							0.7	-0.155	Palomino et al. (1994)
686	RBC	Estradiol 17-acetate							29	1.462	Korenman (1969)
687	RBC	17 -Estradiol 3-acetate	4245-41-4						97	1.987	Korenman (1969)
688	GST-aERdef	Estradiol 3-benzoate	50-50-0		0.024	0.003			13	1.114	Matthews et al. (2000)
689	GST-cERdef	Estradiol 3-benzoate	50-50-0		0.022	0.001			15	1.176	Matthews et al. (2000)
690	GST-hER def	Estradiol 3-benzoate	50-50-0		0.028	0.005			10	1.000	Matthews et al. (2000)

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	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
621	GST-mER def	Estradiol 3-benzoate	50-50-0		0.023	0.002			12	1.079	Matthews et al. (2000)
622	GST-rtERdef	Estradiol 3-benzoate	50-50-0		0.0037	0.0005			9	0.954	Matthews et al. (2000)
623	RBC	Estradiol diacetate	3434-88-6						11	1.041	Korenman (1969)
624	RBC	17 -Estradiol 3-methyl ether	1035-77-4						3	0.477	Korenman (1969)
625	RUC	17 -Estradiol 3-methyl ether	1035-77-4						0.7	-0.155	Gabbard and Segaloff (1983)
626	MCF-7 cells	9-Estratetraene-3,17 -diol	791-69-5						37	1.568	Stoessel and Leclercq (1986)
627	MCF-7 cytosol	9-Estratetraene-3,17 -diol	791-69-5						80	1.903	Stoessel and Leclercq (1986)
628	RBC	Estra-1,3,5(10),6-tetraen-17-one, 3-hydroxy-							10	1.000	Korenman (1969)
629	MCF-7 cells	Estra-1,3,5(10)-triene-3,17 -diol, 14,15 -epoxy-	79581-12-7						10	1.000	Stoessel and Leclercq (1986)
700	MCF-7 cytosol	Estra-1,3,5(10)-triene-3,17 -diol, 14,15 -epoxy-	79581-12-7						5	0.699	Stoessel and Leclercq (1986)
701	MCF-7 cytosol	Estra-1,3,5(10)-triene-3,17 -diol, 14 ,15 -epoxy-	79645-49-1				0.1				Stoessel and Leclercq (1986)
702	MCF-7 cells	Estra-1,3,5(10)-triene-3,17 -diol, 14 ,15 -epoxy-	79645-49-1						0.08	-1.097	Stoessel and Leclercq (1986)
703	MCF-7 cells	Estra-1,3,5,(10),trien-3,14,17 -triol	16288-09-8						1.5	0.176	Stoessel and Leclercq (1986)
704	MCF-7 cytosol	Estra-1,3,5,(10),trien-3,14,17 -triol	16288-09-8						2	0.301	Stoessel and Leclercq (1986)
705	RUC	Estratriene-3,6 ,17 -triol	1229-24-9		0.127	0.043			0.71	-0.150	Blair et al. (2000)
706	GST-aERdef	Estrinol	50-27-1		0.01	0.001			30	1.477	Matthews et al. (2000)
707	GST-cERdef	Estrinol	50-27-1		0.029	0.001			11	1.041	Matthews et al. (2000)
708	GST-hER def	Estrinol	50-27-1		0.01	0.003			28	1.447	Matthews et al. (2000)
709	GST-mER def	Estrinol	50-27-1		0.021	0.005			13	1.114	Matthews et al. (2000)
710	GST-rtERdef	Estrinol	50-27-1		0.09	0.006			3.7	0.568	Matthews et al. (2000)
711	hER	Estrinol	50-27-1					0.0014	14	1.146	Kuiper et al. (1997)
712	MCF-7 cells	Estrinol	50-27-1						20	1.301	Stoessel and Leclercq (1986)
713	MCF-7 cytosol	Estrinol	50-27-1						13	1.114	Brooks et al. (1987)
714	MCF-7 cytosol	Estrinol	50-27-1						18	1.255	Stoessel and Leclercq (1986)
715	MCF-7 cytosol	Estrinol	50-27-1						0.17	-0.770	VanderKuur et al. (1993)
716	MUC	Estrinol	50-27-1						100	2.000	Korach (1979)
717	RBC	Estrinol	50-27-1						16	1.204	Korenman (1969)
718	rtER	Estrinol	50-27-1					0.0007	21	1.320	Kuiper et al. (1997)
719	RUC	Estrinol	50-27-1					0.014	19	1.279	Waller et al. (1996)
720	RUC	Estrinol	50-27-1	99	0.00925	0.00175			9.719	0.990	Blair et al. (2000)
721	GST-aERdef	Estrone	53-16-7		0.0051	0.0001			60	1.778	Matthews et al. (2000)
722	GST-cERdef	Estrone	53-16-7		0.0064	0.0001			50	1.699	Matthews et al. (2000)
723	GST-hER def	Estrone	53-16-7		0.0065	0.0003			45	1.653	Matthews et al. (2000)
724	GST-mER def	Estrone	53-16-7		0.0095	0.0008			28	1.447	Matthews et al. (2000)
725	GST-rtERdef	Estrone	53-16-7		0.024	0.002			14	1.146	Matthews et al. (2000)
726	hER	Estrone	53-16-7					0.0003	60	1.778	Kuiper et al. (1997)
727	hER -FP	Estrone	53-16-7	98	0.626				2.1	0.322	Bolger et al. (1998)
728	MCF-7 cytosol	Estrone	53-16-7						0.22	-0.658	VanderKuur et al. (1993)
729	MUC	Estrone	53-16-7						100	2.000	Korach (1979)
730	rtER	Estrone	53-16-7					0.0004	37	1.568	Kuiper et al. (1997)
731	RUC	Estrone	53-16-7	99	0.0123	0.0032			7.31	0.860	Blair et al. (2000)
732	RUC	Estrone	53-16-7		0.01				50	1.699	Elsby et al. (2000)
733	RUC	Estrone	53-16-7						46	1.663	Gabbard and Segaloff (1983)
734	RUC	Estrone	53-16-7					0.0044	59	1.771	Waller et al. (1996)
735	RBC	Estrone	53-16-7						66	1.820	Korenman (1969)
736	MCF-7 cells	Estrone	53-16-7						19	1.279	Stoessel and Leclercq (1986)
737	MCF-7 cytosol	Estrone	53-16-7						13	1.114	Brooks et al. (1987)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)††	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
738	MCF-7 cytosol	Estrone	53-16-7						15	1.176	Stoessel and Leclercq (1986)
739	RBC	Estrone 3-acetate	901-93-9						191	2.281	Korenman (1969)
740	RUC	Estrone 3-methyl ether	1624-62-0				1				Gabbard and Segaloff (1983)
741	hERα	Estrone 3-sulfate	481-97-0				100				Kuiper et al. (1997)
742	rER	Estrone 3-sulfate	481-97-0				100				Kuiper et al. (1997)
743	GST-aERdef	17 -Ethinyl estradiol	57-63-6		0.0022	0.0001			139	2.143	Matthews et al. (2000)
744	GST-cERdef	17 -Ethinyl estradiol	57-63-6		0.0019	0.0001			171	2.233	Matthews et al. (2000)
745	GST-hER def	17 -Ethinyl estradiol	57-63-6		0.0023	0.0001			127	2.104	Matthews et al. (2000)
746	GST-mER def	17 -Ethinyl estradiol	57-63-6		0.0022	0.0007			118	2.072	Matthews et al. (2000)
747	GST-rERdef	17 -Ethinyl estradiol	57-63-6		0.0031	0.0009			108	2.033	Matthews et al. (2000)
748	RBC	17 -Ethinyl estradiol	57-63-6						191	2.281	Korenman (1969)
749	RUC	17 -Ethinyl estradiol	57-63-6	98	0.004				156	2.180	Allen et al. (1980)
750	RUC	17 -Ethinyl estradiol	57-63-6						190	2.279	Fang et al. (2001)
751	RUC	17 -Ethinyl estradiol	57-63-6		0.0011			0.0004	100	2.000	Laws et al. (2000)
752	RUC	17 -Ethinyl estradiol	57-63-6					0.0002999	867	2.938	Waller et al. (1996)
753	RUC	17 -Ethinyl estradiol	4717-38-8		0.000473	0.00006			190.063	2.280	Blair et al. (2000)
754	RUC	Ethyl cinnamate	103-36-6	99.1			1000				Blair et al. (2000)
755	RUC	3-Ethyl-6,4'-dihydroxy-2-phenylindene							16	1.204	Anstead et al. (1989)
756	RUC	2-Ethylhexyl paraben	5153-25-3	99	4.95	0.05			0.018	-1.740	Blair et al. (2000)
757	RUC	4-Ethyl-7-hydroxy-3-(4-methoxyphenyl)-2H -1-benzopyran-2-one	5219-17-0						0.9	-0.460	Fang et al. (2001)
758	RUC	3-[(10-Ethyl-11- <i>p</i> -hydroxyphenyl)dibenzo- <i>[b,f]</i>]oxepin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)	85850-93-7						0.92	-0.036	Acton et al. (1983)
759	RUC	3-[(10-Ethyl-11- <i>p</i> -hydroxyphenyl)dibenzo- <i>[b,f]</i>]thiepin-3-yl)oxy]-1,2-propanediol	85850-94-8						11.0	1.041	Acton et al. (1983)
760	RUC	3-[(11-Ethyl-12-(<i>p</i> -hydroxyphenyl)-6- <i>H</i> -dibenzo- <i>[b,f]</i>]thiicin-3-yl)oxy]-, hemihydrate 1,2-propanediol	85864-54-6						5.0	0.699	Acton et al. (1983)
761	RUC	3-[(6-Ethyl-5-(<i>p</i> -hydroxyphenyl)-11,12-dihydrodibenzo- <i>[a,e]</i>]cycloocten-2-yl)oxy]-1,2-propanediol	85850-95-9						9.1	0.959	Acton et al. (1983)
762	RUC	3-Ethyl-4'-hydroxy-2-phenylindene							2.3	0.362	Anstead et al. (1989)
763	RUC	3-Ethyl-6-hydroxy 2-phenylindene							0.58	-0.237	Anstead et al. (1989)
764	RUC	3-Ethyl-4'-hydroxy 2-phenylindenone-1							4.6	0.663	Anstead et al. (1989)
765	RUC	3-Ethyl-6-hydroxy 2-phenylindenone-1							1.2	0.079	Anstead et al. (1989)
766	RBC	3-Ethyl-4-(<i>p</i> -methoxyphenyl)-2-methyl-3-cyclohexene-1-carboxylic acid	1755-52-8						0.75	-0.125	Korenman (1969)
767	RUC	Ethyl paraben	120-47-8	99	150	10			0.0006	-3.220	Blair et al. (2000)
768	RUC	2-Ethylphenol	90-00-6	99			1000				Blair et al. (2000)
769	RUC	3-Ethylphenol	620-17-7	80	660	76			0.00014	-3.87	Blair et al. (2000)
770	RUC	4-Ethylphenol	123-07-9	99	1340	40			0.00007	-4.170	Blair et al. (2000)
771	RUC	3-[(10-Ethyl-11-phenyldibenzo- <i>[b,f]</i>]thiepin-3-yl)oxy]-1,2-propanediol, complexed with isopropyl alcohol 2:1	85850-90-4						0.65	-0.187	Acton et al. (1983)
772	RUC	3-[(11-Ethyl-12-phenyl-6- <i>H</i> -dibenzo- <i>[b,f]</i>]thiicin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)	85850-92-6						0.02	-1.699	Acton et al. (1983)
773	RUC	3-[6-Ethyl-5-phenyl-11,12-dihydrodibenzo- <i>[a,e]</i>]cycloocten-2-yl)oxy]-1,2-propanediol	85850-91-5						0.12	-0.921	Acton et al. (1983)
774	RUC	Eugenol	97-53-0	99.2			1000				Blair et al. (2000)
775	hER -FP	Fenvalerate	51630-58-1	> 93			10				Saito et al. (2000)
776	RUC	Fisetin	528-48-3						0.0045	2.350	Fang et al. (2001)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)††	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
777	RUC	Flavanone	17002-31-2				100				Fang et al. (2001)
778	hER	Flavone	525-82-6				10				Kuiper et al. (1998) [method a]
779	hER	Flavone	525-82-6				10				Kuiper et al. (1998) [method a]
780	RUC	Flavone	525-82-6				100				Fang et al. (2001)
781	MCF-7 cells	Fluoranthene	206-44-0				5				Arcaro et al. (1999)
782	MCF-7 cells	Fluorene	86-73-7				5				Arcaro et al. (1999)
783	MCF-7 cytosol	2-Fluoroestratrien-17 -ol	101772-22-9						2	0.301	Brooks et al. (1987)
784	MCF-7 cytosol	4-Fluoroestratrien-17 -ol	96607-54-4						8	0.903	Brooks et al. (1987)
785	RUC	2-(2-Fluorophenyl)-3-phenyl-6-hydroxyindene							49	1.690	Anstead et al. (1990)
786	RUC	Fluorotamoxifen	73617-96-6				10				Allen et al. (1980)
787	RUC	Folic acid	59-30-3				100				Blair et al. (2000)
788	hER	Formononetin	485-72-3				10				Kuiper et al. (1998) [method a]
789	hER	Formononetin	485-72-3				10				Kuiper et al. (1998) [method a]
790	RUC	Formononetin	485-72-3						0.0013	-2.890	Fang et al. (2001)
791	RUC	Furfural	98-01-1	99.4			1000				Blair et al. (2000)
792	GST-aERdef	Genistein	446-72-0		0.24	0.01			1.3	0.114	Matthews et al. (2000)
793	GST-cERdef	Genistein	446-72-0		0.41	0.05			0.78	-0.108	Matthews et al. (2000)
794	GST-hER def	Genistein	446-72-0		0.063	0.07			0.46	-0.337	Matthews et al. (2000)
795	GST-mER def	Genistein	446-72-0		0.81	0.04			0.33	-0.481	Matthews et al. (2000)
796	GST-rERdef	Genistein	446-72-0		0.75	0.08			0.44	-0.357	Matthews et al. (2000)
797	hER	Genistein	446-72-0					0.0026	5	0.699	Kuiper et al. (1997)
798	hER	Genistein	446-72-0						4	0.602	Kuiper et al. (1998) [method a]
799	hER	Genistein	446-72-0		0.145				0.7	-0.155	Kuiper et al. (1998) [method b]
800	hER	Genistein	446-72-0		0.7				0.71	-0.15	Morito et al. (2001)
801	hER -FP	Genistein	446-72-0		0.825	0.002			1.6	0.204	Nikov et al. (2000)
802	hER	Genistein	446-72-0						87	1.940	Kuiper et al. (1998) [method a]
803	hER	Genistein	446-72-0		0.0084				13	1.114	Kuiper et al. (1998) [method b]
804	hER	Genistein	446-72-0		0.011				45.5	1.66	Morito et al. (2001)
805	MCF-7 cytosol	Genistein	446-72-0		0.045				2	0.301	Dodge et al. (1996)
806	MCF-7 cytosol	Genistein	446-72-0		0.23				2.61	0.417	Miodini et al. (1999)
807	rER	Genistein	446-72-0					0.0003	36	1.556	Kuiper et al. (1997)
808	RUC	Genistein	446-72-0						0.45	-0.350	Fang et al. (2001)
809	RUC	Genistein	446-72-0					0.39	0.67	-0.180	Waller et al. (1996)
810	hER	Genistin	529-59-9		37				0.014	-1.87	Morito et al. (2001)
811	RUC	Genistin	529-59-9				100				Fang et al. (2001)
812	hER -FP	Glyceollin	66241-09-6		6	0.6			0.22	-0.658	Nikov et al. (2000)
813	hER	Glycitein	40957-83-3		32				0.016	-1.81	Morito et al. (2001)
814	hER	Glycitein	40957-83-3		0.55				0.91	-0.04	Morito et al. (2001)
815	hER	Glycitin			650				0.0008	-3.10	Morito et al. (2001)
816	RUC	Heptachlor	76-44-8	99.5			100				Blair et al. (2000)
817	GST-aERdef	2,2',3,3',4',5',6'-Heptachlorobiphenyl	52663-70-4				10				Matthews and Zacharewski (2000)
818	GST-hER def	2,2',3,3',4',5',6'-Heptachlorobiphenyl	52663-70-4				10				Matthews and Zacharewski (2000)
819	GST-rERdef	2,2',3,3',4',5',6'-Heptachlorobiphenyl	52663-70-4		10				0.031	-1.509	Matthews and Zacharewski (2000)
820	GST-aERdef	2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1				10				Matthews and Zacharewski (2000)
821	GST-hER def	2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1				10				Matthews and Zacharewski (2000)
822	GST-rERdef	2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1		10				0.031	-1.509	Matthews and Zacharewski (2000)
823	GST-aERdef	2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6				10				Matthews and Zacharewski (2000)
824	GST-hERadef	2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6				10				Matthews and Zacharewski (2000)

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1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
825	GST-rtERdef	2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6				10				Matthews and Zacharewski (2000)
826	GST-aERdef	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1				10				Matthews and Zacharewski (2000)
827	GST-hER def	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1				10				Matthews and Zacharewski (2000)
828	GST-rtERdef	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1				10				Matthews and Zacharewski (2000)
829	GST-aERdef	2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3		10				0.025	-1.602	Matthews and Zacharewski (2000)
830	GST-hER def	2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3		10				0.024	-1.620	Matthews and Zacharewski (2000)
831	GST-rtERdef	2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3		0.4	0.1			0.78	-0.111	Matthews and Zacharewski (2000)
832	GST-aERdef	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0				10				Matthews and Zacharewski (2000)
833	GST-hER def	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0				10				Matthews and Zacharewski (2000)
834	GST-rtERdef	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0				10				Matthews and Zacharewski (2000)
835	GST-aERdef	2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7		10				0.025	-1.602	Matthews and Zacharewski (2000)
836	GST-hER def	2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7		10				0.024	-1.620	Matthews and Zacharewski (2000)
837	GST-rtERdef	2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7		1.3	1.2			0.24	-0.623	Matthews and Zacharewski (2000)
838	GST-aERdef	2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7				10				Matthews and Zacharewski (2000)
839	GST-hER def	2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7				10				Matthews and Zacharewski (2000)
840	GST-rtERdef	2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7				10				Matthews and Zacharewski (2000)
841	GST-aERdef	2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8				10				Matthews and Zacharewski (2000)
842	GST-hER def	2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8				10				Matthews and Zacharewski (2000)
843	GST-rtERdef	2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8				10				Matthews and Zacharewski (2000)
844	hER	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
845	hER	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
846	GST-aERdef	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3				10				Matthews and Zacharewski (2000)
847	GST-hER def	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3				10				Matthews and Zacharewski (2000)
848	GST-rtERdef	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3				10				Matthews and Zacharewski (2000)
849	hER	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	> 98					0.09	-1.046	Kuiper et al. (1998) [method a]
850	hER	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	> 98					0.09	-1.046	Kuiper et al. (1998) [method a]
851	hER	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
852	hER	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
853	GST-aERdef	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8				10				Matthews and Zacharewski (2000)
854	GST-hER def	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8				10				Matthews and Zacharewski (2000)
855	GST-rtERdef	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8				10				Matthews and Zacharewski (2000)
856	hER	2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
857	hER	2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
858	RUC	Heptanal	111-71-7	92.9			10000				Blair et al. (2000)
859	RUC	4-(Heptyloxy)phenol	13037-86-0	97	67.5	7.5			0.0013	-2.880	Blair et al. (2000)
860	RUC	Heptyl 4-paraben	1085-12-7	97	11	1			0.008	-2.090	Blair et al. (2000)
861	RUC	Hesperetin	520-33-2				100				Fang et al. (2001)
862	RUC	Hexachlorobenzene	118-74-1				1000				Blair et al. (2000)
863	GST-aERdef	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3				10				Matthews and Zacharewski (2000)
864	GST-hER def	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3				10				Matthews and Zacharewski (2000)
865	GST-rtERdef	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3				10				Matthews and Zacharewski (2000)
866	GST-aERdef	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2				10				Matthews and Zacharewski (2000)
867	GST-hER def	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2				10				Matthews and Zacharewski (2000)
868	GST-rtERdef	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2				10				Matthews and Zacharewski (2000)
869	GST-aERdef	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0				10				Matthews and Zacharewski (2000)
870	GST-hER def	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0				10				Matthews and Zacharewski (2000)
871	GST-rtERdef	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0				10				Matthews and Zacharewski (2000)
872	GST-aERdef	2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0				10				Matthews and Zacharewski (2000)
873	GST-hER def	2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0				10				Matthews and Zacharewski (2000)

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874	GST-rtERdef	2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0		10				0.031	-1.509	Matthews and Zacharewski (2000)
875	GST-aERdef	2,2',3,5,5',6'-Hexachlorobiphenyl	52663-63-5				10				Matthews and Zacharewski (2000)
876	GST-hER def	2,2',3,5,5',6'-Hexachlorobiphenyl	52663-63-5				10				Matthews and Zacharewski (2000)
877	GST-rtERdef	2,2',3,5,5',6'-Hexachlorobiphenyl	52663-63-5				10				Matthews and Zacharewski (2000)
878	GST-aERdef	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1				10				Matthews and Zacharewski (2000)
879	GST-hER def	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1				10				Matthews and Zacharewski (2000)
880	GST-rtERdef	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1				10				Matthews and Zacharewski (2000)
881	hER	2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2	≥ 99%			50				Vakharia and Gierthy (2000)
882	MUC	2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2	> 98	5.6				0.27	-0.569	Fielden et al. (1997)
883	GST-aERdef	2,3,3',4,4',6'-Hexachlorobiphenyl	74472-42-7				10				Matthews and Zacharewski (2000)
884	GST-hERadef	2,3,3',4,4',6'-Hexachlorobiphenyl	74472-42-7				10				Matthews and Zacharewski (2000)
885	GST-rtERdef	2,3,3',4,4',6'-Hexachlorobiphenyl	74472-42-7				10				Matthews and Zacharewski (2000)
886	GST-aERdef	2,3',4,4',5',6'-Hexachlorobiphenyl	59291-65-5				10				Matthews and Zacharewski (2000)
887	GST-hER def	2,3',4,4',5',6'-Hexachlorobiphenyl	59291-65-5				10				Matthews and Zacharewski (2000)
888	GST-rtERdef	2,3',4,4',5',6'-Hexachlorobiphenyl	59291-65-5				10				Matthews and Zacharewski (2000)
889	GST-aERdef	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6				10				Matthews and Zacharewski (2000)
890	GST-hER def	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6				10				Matthews and Zacharewski (2000)
891	GST-rtERdef	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6				10				Matthews and Zacharewski (2000)
892	hER	2,2',3,3',4',5'-Hexachloro-4-biphenylol	158076-62-1	> 98					0.07	-1.155	Kuiper et al. (1998) [method a]
893	hER	2,2',3,3',4',5'-Hexachloro-4-biphenylol	158076-62-1	> 98					0.06	-1.222	Kuiper et al. (1998) [method a]
894	GST-aERdef	2,2',3,3',4',5'-Hexachloro-4-biphenylol	158076-62-1				10				Matthews and Zacharewski (2000)
895	GST-hER def	2,2',3,3',4',5'-hexachloro-4-biphenylol	158076-62-1				10				Matthews and Zacharewski (2000)
896	GST-rtERdef	2,2',3,3',4',5'-Hexachloro-4-biphenylol	158076-62-1				10				Matthews and Zacharewski (2000)
897	GST-aERdef	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7				10				Matthews and Zacharewski (2000)
898	GST-hER def	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7				10				Matthews and Zacharewski (2000)
899	GST-rtERdef	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7				10				Matthews and Zacharewski (2000)
900	hER	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	> 98					0.03	-1.523	Kuiper et al. (1998) [method a]
901	hER	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	> 98					0.04	-1.398	Kuiper et al. (1998) [method a]
902	MCF-7 cytosol	2',3,3',4',5,5'-Hexachloro-4-biphenylol	158076-63-2	> 95	2.8				3.2	0.505	Kramer et al. (1997)
903	RUC	n-Hexanol	111-27-3	98.9			10000				Blair et al. (2000)
904	hER	Hexestrol	84-16-2					0.00006	302	2.480	Kuiper et al. (1997)
905	MCF-7 cells	Hexestrol	84-16-2						58	1.763	Stoessel and Leclercq (1986)
906	MCF-7 cytosol	Hexestrol	84-16-2						100	2.000	Stoessel and Leclercq (1986)
907	RBC	Hexestrol	84-16-2						74	1.869	Korenman (1969)
908	rER	Hexestrol	84-16-2					0.00006	234	2.369	Kuiper et al. (1997)
909	RUC	Hexestrol	84-16-2	99	0.0003	0			299.67	2.480	Blair et al. (2000)
910	RUC	Hexestrol	84-16-2						300	2.477	Fang et al. (2001)
911	RUC	DL -Hexestrol	5776-72-7		0.025	0.005			3.60	0.560	Blair et al. (2000)
912	RUC	Hexestrol monomethyl ether	13026-26-1		0.0096	0.0014			9.37	0.970	Blair et al. (2000)
913	GST-hER def	3-Hydroxybenzo[b]naphtho[2,1-d]thiophene			0.3	0.074			1.83	0.26	Fertuck et al. (2001)
914	hER	3-Hydroxybenzo[b]naphtho[2,1-d]thiophene			0.22	0.082			2.50	0.40	Fertuck et al. (2001)
915	GST-hER def	2-Hydroxybenzo-[c]phenanthrene	22717-94-8		0.25	0.004			2.2	0.34	Fertuck et al. (2001)
916	hER	2-Hydroxybenzo-[c]phenanthrene	22717-94-8		0.18	0.1			3.10	0.49	Fertuck et al. (2001)
917	GST-hER def	3-Hydroxybenzo-[b]phenanthro[2,3-d]thiophene			0.23	0.01			2.40	0.38	Fertuck et al. (2001)
918	hER	3-Hydroxybenzo-[b]phenanthro[2,3-d]thiophene			0.11	0.038			5.0	0.70	Fertuck et al. (2001)
919	RUC	4-Hydroxychalcone	20426-12-4						0.0028	-2.430	Fang et al. (2001)
920	RUC	4'-Hydroxychalcone	2657-25-2						0.0037	-2.430	Fang et al. (2001)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
921	RUC	4'-Hydroxychalcone (<i>cis</i> - and <i>trans</i> -)	38239-52-0						0.0037	-2.550	Fang et al. (2001)
922	GST-hER def	2-Hydroxychrysene	65945-06-4		0.095	0.044			5.80	0.76	Fertuck et al. (2001)
923	hER	2-Hydroxychrysene	65945-06-4		0.042	0.014			13.33	1.12	Fertuck et al. (2001)
924	RUC	4'-Hydroxy-2,3-diphenylnone-1							0.45	-0.347	Anstead et al. (1989)
925	RUC	6'-Hydroxy-2,3-diphenylnone-1							59	1.771	Anstead et al. (1989)
926	MCF-7 cytosol	11 -Hydroxyestradiol	1464-61-5						3.1	0.491	Palomino et al. (1994)
927	RBC	11 -Hydroxyestradiol	5444-22-4						4	0.602	Korenman (1969)
928	MCF-7 cytosol	11 -Hydroxyestradiol	5444-22-4						16.8	1.225	Palomino et al. (1994)
929	RUC	11 -Hydroxyestradiol	5444-22-4						7	0.845	Qian and Abul-Hajj (1990)
930	MCF-7 cells	11 -Hydroxyestradiol	5444-22-4						4	0.602	Stoessel and Leclercq (1986)
931	MCF-7 cytosol	11 -Hydroxyestradiol	5444-22-4						1	0.000	Stoessel and Leclercq (1986)
932	MCF-7 cells	14 -Hydroxyestradiol	60183-66-6						3.5	0.544	Stoessel and Leclercq (1986)
933	MCF-7 cytosol	14 -Hydroxyestradiol	60183-66-6						10	1.000	Stoessel and Leclercq (1986)
934	hER	2-Hydroxyestradiol	362-05-0					0.0025	7	0.845	Kuiper et al. (1997)
935	rER	2-Hydroxyestradiol	362-05-0					0.0013	11	1.040	Kuiper et al. (1997)
936	hER	4-Hydroxyestradiol	5976-61-4					0.001	13	1.114	Kuiper et al. (1997)
937	rER	4-Hydroxyestradiol	5976-61-4					0.0019	7	0.845	Kuiper et al. (1997)
938	MCF-7 cytosol	2-Hydroxyestratrien-17 -ol	2259-89-4						18	1.255	Brooks et al. (1987)
939	MCF-7 cytosol	4-Hydroxyestratrien-17 -ol	17592-89-1						0.8	-0.097	Brooks et al. (1987)
940	RUC	3-Hydroxyestra-1,3,5(10)-trien-16-one	3601-97-6		0.175	0.005			0.51	-0.290	Blair et al. (2000)
941	hER	2-Hydroxyestrone	362-06-1						2	0.301	Kuiper et al. (1998) [method a]
942	hER	2-Hydroxyestrone	362-06-1						0.2	-0.699	Kuiper et al. (1998) [method a]
943	hER -FP	2-Hydroxyethyl methacrylate	868-77-9	95			5000				Hashimoto et al. (2000)
944	RUC	3'-Hydroxyflavanone	92496-65-6						0.0017	-2.770	Fang et al. (2001)
945	RUC	4'-Hydroxyflavanone	135413-27-3						0.0023	-2.640	Fang et al. (2001)
946	RUC	6-Hydroxyflavanone	4250-77-5						0.0009	-3.050	Fang et al. (2001)
947	RUC	7-Hydroxyflavanone	6515-36-2						0.00019	-3.720	Fang et al. (2001)
948	RUC	6-Hydroxyflavone	6665-83-4						0.0004	-3.398	Fang et al. (2001)
949	RUC	7-Hydroxyflavone	6665-86-7				100				Fang et al. (2001)
950	RUC	Hydroxyflutamide	52806-53-8				1000				Laws et al. (1996)
951	RUC	Hydroxyflutamide	52806-53-8					1000	0.0003	-3.523	Waller et al. (1996)
952	RUC	2-Hydroxy-4-methoxybenzophenone	131-57-7	98			100				Blair et al. (2000)
953	RUC	6-Hydroxy-2'-methoxyflavone	61546-59-6				100				Fang et al. (2001)
954	GST-hER def	2-Hydroxy-5-methylchrysene			0.028	0.012			19.60	1.29	Fertuck et al. (2001)
955	hER	2-Hydroxy-5-methylchrysene			0.029	0.005			19.30	1.29	Fertuck et al. (2001)
956	GST-hER def	8-Hydroxy-5-methylchrysene			0.18	0.026			3.10	0.49	Fertuck et al. (2001)
957	hER	8-Hydroxy-5-methylchrysene			0.18	0.032			3.10	0.49	Fertuck et al. (2001)
958	RUC	16 -Hydroxy-16-methyl-17 -estradiol 3-methyl ether	3434-79-5		2.7	0.2			0.033	-1.480	Blair et al. (2000)
959	hER	4-Hydroxytamoxifen	68047-06-3					0.0001	178	2.250	Kuiper et al. (1997)
960	hER	4-Hydroxytamoxifen	68047-06-3						257	2.410	Kuiper et al. (1998) [method a]
961	hER	4-Hydroxytamoxifen	68047-06-3					0.00022	149	2.173	Sun et al. (1999)
962	hER -FP	4-Hydroxytamoxifen	68047-06-3	> 93	0.01				400	2.602	Saito et al. (2000)
963	hER	4-Hydroxytamoxifen	68047-06-3						232	2.365	Kuiper et al. (1998) [method a]
964	hER	4-Hydroxytamoxifen	68047-06-3					0.0015	62	1.792	Sun et al. (1999)
965	MUC	4-Hydroxytamoxifen	68047-06-3	> 99	0.0125				14.4	1.150	Shelby et al. (1996)
966	rER	4-Hydroxytamoxifen	68047-06-3					0.00004	339	2.530	Kuiper et al. (1997)
967	GST-aERdef	4-Hydroxytamoxifen	68047-06-3		0.0013	0.0001			243	2.386	Matthews et al. (2000)
968	GST-cERdef	4-Hydroxytamoxifen	68047-06-3		0.0019	0.0003			168	2.225	Matthews et al. (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
969	GST-hER def	4-Hydroxytamoxifen	68047-06-3		0.0019	0.0001			155	2.190	Matthews et al. (2000)
970	GST-mER def	4-Hydroxytamoxifen	68047-06-3		0.0012	0.0004			212	2.326	Matthews et al. (2000)
971	GST-rtERdef	4-Hydroxytamoxifen	68047-06-3		0.0012	0.0009			272	2.435	Matthews et al. (2000)
972	hER -FP	4-Hydroxytamoxifen	68047-06-3		0.096	0.0008			14	1.146	Nikov et al. (2001)
973	hER -FP	4-Hydroxytamoxifen	68047-06-3		0.026				21.5	1.332	Parker et al. (2000)
974	RUC	4-Hydroxytamoxifen	68047-06-3		0.000513	0.000112			175.244	2.240	Blair et al. (2000)
975	MCF-7 cells	4-Hydroxytamoxifen	68047-06-3						2.9	0.462	Stoessel and Leclercq (1986)
976	MCF-7 cytosol	4-Hydroxytamoxifen	68047-06-3						100	2.000	Stoessel and Leclercq (1986)
977	RUC	6-Hydroxytetralin	1125-78-6				500				Elsby et al. (2000)
978	GST-aERdef	ICI 164384	98007-99-9		0.011	0.002			28	1.447	Matthews et al. (2000)
979	GST-cERdef	ICI 164384	98007-99-9		0.0052	0.001			62	1.792	Matthews et al. (2000)
980	GST-hER def	ICI 164384	98007-99-9		0.007	0.0003			42	1.623	Matthews et al. (2000)
981	GST-mER def	ICI 164384	98007-99-9		0.0059	0.0003			45	1.653	Matthews et al. (2000)
982	GST-rtERdef	ICI 164384	98007-99-9		0.001	0.0007			327	2.515	Matthews et al. (2000)
983	hER	ICI 164384	98007-99-9					0.0002	85	1.929	Kuiper et al. (1997)
984	hER	ICI 164384	98007-99-9		0.00059				158.0	2.200	Kuiper et al. (1998) [method b]
985	hER	ICI 164384	98007-99-9		0.00114				93.0	1.970	Kuiper et al. (1998) [method b]
986	rER	ICI 164384	98007-99-9					0.00008	166	2.220	Kuiper et al. (1997)
987	RUC	ICI 164384	98007-99-9		0.0062	0.0013			14.5	1.160	Blair et al. (2000)
988	hER	ICI 182780	129453-61-8					0.001	32	1.505	Sun et al. (1999)
989	hER	ICI 182780	129453-61-8					0.0036	25	1.398	Sun et al. (1999)
990	RBC	ICI 182780	129453-61-8	99.3	0.000004				500	2.699	Andersen et al. (1999)
991	RUC	ICI 182780	129453-61-8		0.0024	0.0011			37.46	1.570	Blair et al. (2000)
992	RUC	ICI 182780	129453-61-8					0.00059979	433	2.636	Waller et al. (1996)
993	hER -FP	Imiprophrin	72963-72-5	> 93			10				Saito et al. (2000)
994	MUC	Indanestrol	71855-45-3		0.05	0.005			2	0.301	Korach et al. (1979)
995	MUC	Indanestrol	71855-45-3		6	5			2.67	0.427	Korach et al. (1985)
996	MUC	Indanyldiethylstilbestrol			0.002	0.0004			80	1.900	Korach et al. (1978)
997	MUC	Indenestrol A	24643-97-8		0.0007	0.0001			143	2.155	Korach et al. (1979)
998	MUC	Indenestrol A	24643-97-8		0.07	0.1			229	2.360	Korach et al. (1985)
999	MUC	(R)-Indenestrol A	115217-03-3						13	1.110	Korach et al. (1989)
1000	MUC	(rac)-Indenestrol A	115217-02-2						143	2.155	Korach et al. (1989)
1001	MUC	(S)-Indenestrol A	115217-04-4						285	2.460	Korach et al. (1989)
1002	MUC	Indenestrol B	38028-27-2		0.0007	0.0002			143	2.155	Korach et al. (1979)
1003	MUC	Indenestrol B	38028-27-2		0.7	0.1			229	2.360	Korach et al. (1985)
1004	MUC	(R)-Indenestrol B	115217-06-6						100	2.000	Korach et al. (1989)
1005	MUC	(rac) Indenestrol B	133830-97-4						145	2.160	Korach et al. (1989)
1006	MUC	(S)-Indenestrol B							143	2.160	Korach et al. (1989)
1007	MCF-7 cells	Indeno[1,2,3- <i>cd</i>]pyrene	193-39-5						20	1.301	Arcaro et al. (1999)
1008	RUC	Indole[3,2- <i>c</i>]carbazole		> 98	23				0.00083	-3.081	Liu et al. (1994)
1009	MCF-7 cytosol	16-Iodoestradiol	71765-94-1		0.006				100	2.000	Miodini et al. (1999)
1010	MCF-7 cytosol	(E)-17-Iodovinylestradiol	82123-96-4					0.00104	17	1.230	Rijks et al. (1996)
1011	RUC	(E)-17-Iodovinylestradiol	82123-96-4					0.0022	7	0.845	Rijks et al. (1996)
1012	MCF-7 cytosol	(Z)-17-Iodovinylestradiol	177159-09-0					0.00039	51	1.708	Rijks et al. (1996)
1013	RUC	(Z)-17-Iodovinylestradiol	177159-09-0					0.00025	63	1.799	Rijks et al. (1996)
1014	hER	Ipriflavone	35212-22-7				10				Kuiper et al. (1998) [method a]
1015	hER	Ipriflavone	35212-22-7				10				Kuiper et al. (1998) [method a]
1016	RUC	Isoeugenol	97-54-1	98			100				Blair et al. (2000)
1017	hER	Kaempferol	520-18-3						0.1	-1.000	Kuiper et al. (1998) [method a]

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1018	hER	Kaempferol	520-18-3						3	0.477	Kuiper et al. (1998) [method a]
1019	hER	Kaempferol	520-18-3		0.054				2	0.301	Kuiper et al. (1998) [method b]
1020	RUC	Kaempferol	520-18-3						0.025	-1.600	Fang et al. (2001)
1021	GST-aERdef	Kepone	143-50-0		27	7			0.011	-1.959	Matthews et al. (2000)
1022	GST-cERdef	Kepone	143-50-0		30	1			0.011	-1.959	Matthews et al. (2000)
1023	GST-hER def	Kepone	143-50-0		42	18			0.0069	-2.161	Matthews et al. (2000)
1024	GST-mER def	Kepone	143-50-0		64	3			0.0035	-2.456	Matthews et al. (2000)
1025	GST-rERdef	Kepone	143-50-0		6.2	0.4			0.054	-1.268	Matthews et al. (2000)
1026	hER	Kepone	143-50-0						0.06	-1.222	Kuiper et al. (1998) [method a]
1027	hER -FP	Kepone	143-50-0	87.5	5.7				0.2	-0.699	Bolger et al. (1998)
1028	hER	Kepone	143-50-0						0.1	-1.000	Kuiper et al. (1998) [method a]
1029	MUC	Kepone	143-50-0	98			5				Shelby et al. (1996)
1030	RUC	Kepone	143-50-0		7.0	1.00			0.013	-1.890	Blair et al. (2000)
1031	RUC	Kepone	143-50-0		4.005			1.5	0.03	-0.570	Laws et al. (2000)
1032	RUC	Kepone	143-50-0					1.40	0.1862	-0.730	Waller et al. (1996)
1033	MCF-7 cytosol	11-Keto-9 -estradiol					3				Palomino et al. 1994
1034	hER	16-Ketoestradiol	566-75-6						1.3	0.000	Kuiper et al. (1998) [method a]
1035	hER	16-Ketoestradiol	566-75-6						0.9	-0.046	Kuiper et al. (1998) [method a]
1036	MCF-7 cytosol	16-Ketoestradiol	566-75-6						0.9	-0.046	Palomino et al. (1994)
1037	RBC	16-Ketoestradiol	566-75-6						14	1.146	Korenman (1969)
1038	MCF-7 cells	6-Ketoestradiol	571-92-6						15	1.176	Stoessel and Leclerq (1986)
1039	MCF-7 cytosol	6-Ketoestradiol	571-92-6						20	1.301	Stoessel and Leclerq (1986)
1040	RUC	Lindane	58-89-9	99			100				Blair et al. (2000)
1041	RUC	Lindane	58-89-9					1000	0.0003	-3.523	Waller et al. (1996)
1042	RUC	Melatonin	73-31-4	97			100				Blair et al. (2000)
1043	RBC	MER-25	67-98-1						0.00096	-3.018	Korenman (1970)
1044	RUC	Mestilbol	18839-90-2		0.0044	0.0005			20.43	1.310	Blair et al. (2000)
1045	RBC	Mestranol	72-33-3						8	0.903	Korenman (1969)
1046	RUC	Mestranol	72-33-3	98	2.5				0.25	-0.541	Allen et al. (1980)
1047	RUC	Mestranol	72-33-3		0.0397	0.0065			2.26	0.350	Blair et al. (2000)
1048	GST-aERdef	<i>p,p'</i> - Methoxychlor	72-43-5				100				Matthews et al. (2000)
1049	GST-cERdef	<i>p,p'</i> - Methoxychlor	72-43-5				100				Matthews et al. (2000)
1050	GST-hER def	<i>p,p'</i> - Methoxychlor	72-43-5				100				Matthews et al. (2000)
1051	GST-mER def	<i>p,p'</i> - Methoxychlor	72-43-5				100				Matthews et al. (2000)
1052	GST-rERdef	<i>p,p'</i> - Methoxychlor	72-43-5		3.5	0.4			0.95	-0.022	Matthews et al. (2000)
1053	hER	<i>p,p'</i> - Methoxychlor	72-43-5					1.77	0.01	-2.000	Kuiper et al. (1997)
1054	hER	<i>p,p'</i> - Methoxychlor	72-43-5				10				Kuiper et al. (1998) [method a]
1055	hER -FP	<i>p,p'</i> - Methoxychlor	72-43-5	98	135				0.0096	-2.018	Bolger et al. (1998)
1056	hER	<i>p,p'</i> - Methoxychlor	72-43-5				10				Kuiper et al. (1998) [method a]
1057	MCF-7 cytosol	<i>p,p'</i> - Methoxychlor	72-43-5				1000				Dodge et al. (1996)
1058	MUC	<i>p,p'</i> - Methoxychlor	72-43-5	98			5				Shelby et al. (1996)
1059	RBC	<i>p,p'</i> - Methoxychlor	72-43-5	98.4	6.5				0.0031	-2.509	Andersen et al. (1999)
1060	rER	<i>p,p'</i> - Methoxychlor	72-43-5					0.09	0.13	-0.886	Kuiper et al. (1997)
1061	RUC	<i>p,p'</i> - Methoxychlor	72-43-5	95	144	66			0.001	-3.200	Blair et al. (2000)
1062	RUC	<i>p,p'</i> - Methoxychlor	72-43-5	99			100				Blair et al. (2000)
1063	RUC	<i>p,p'</i> - Methoxychlor	72-43-5	95	174			65	0.00062	-3.210	Laws et al. (2000)
1064	RUC	<i>p,p'</i> - Methoxychlor	72-43-5				100				Nelson (1974)
1065	RUC	<i>p,p'</i> - Methoxychlor	72-43-5					69.02	0.0038	-2.420	Waller et al. (1996)
1066	RUC	Methoxychlor olefin	2132-70-9	95			100				Blair et al. (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1067	RUC	3-Methoxyestradiol	1474-53-9		4	0			0.022	-1.650	Blair et al. (2000)
1068	MCF-7 cytosol	E -11 -methoxy-17 -iodovinylestradiol	90857-55-9					0.00104	17	1.230	Rijks et al. (1996)
1069	RUC	E -11 -methoxy-17 -iodovinylestradiol	90857-55-9					0.0014	11	1.041	Rijks et al. (1996)
1070	MCF-7 cytosol	(Z)-11 -methoxy-17 -iodovinylestradiol	177159-11-4					0.00059	31	1.491	Rijks et al. (1996)
1071	RUC	(Z)-11 -methoxy-17 -iodovinylestradiol	177159-11-4					0.0004	41	1.613	Rijks et al. (1996)
1072	RUC	3-Methoxy-10-methyl-11-phenyldibenzol[<i>b,f</i>]thiepin (16)	85807-06-1						0.005	-2.301	Acton et al. (1983)
1073	RUC	Methoxytamoxifen			0.85				0.74	0.735	Allen et al. (1980)
1074	RUC	7 -Methyl-14-dehydroestradiol-17	88598-62-3						74	1.869	Gabbard and Segaloff (1983)
1075	RUC	9 -Methyl-14-dehydroestradiol-17	88598-63-4						41	1.613	Gabbard and Segaloff (1983)
1076	RUC	7 -Methyl-14-dehydroestradiol-17 3-methyl ether	35644-59-8						3.1	0.491	Gabbard and Segaloff (1983)
1077	RUC	9 -Methyl-14-dehydroestradiol-17 3-methyl ether	88598-64-5						0.1	-1.000	Gabbard and Segaloff (1983)
1078	RUC	11 -Methyl-14-dehydroestradiol-17 3-methyl ether	88598-65-6						1.2	0.079	Gabbard and Segaloff (1983)
1079	RUC	7 -Methyl-14-dehydroestrone	88958-66-7						52	1.716	Gabbard and Segaloff (1983)
1080	RUC	9 -Methyl-14-dehydroestrone	88598-67-8						6	0.778	Gabbard and Segaloff (1983)
1081	RUC	7 -Methyl-14-dehydroestrone 3-methyl ether	35644-57-6				1				Gabbard and Segaloff (1983)
1082	RUC	9 -Methyl-14-dehydroestrone 3-methyl ether					1				Gabbard and Segaloff (1983)
1083	RUC	11 -Methyl-14-dehydroestrone 3-methyl ether	88598-69-0				1				Gabbard and Segaloff (1983)
1084	RUC	4,4'-Methylenebis(N,N-dimethylaniline)	101-61-1	98			1000				Blair et al. (2000)
1085	RUC	4,4'-Methylenedianiline	101-77-9	97			233				Blair et al. (2000)
1086	RUC	7 -Methylestradiol-17	10448-97-2						104	2.017	Gabbard and Segaloff (1983)
1087	RUC	9 -Methylestradiol-17	66463-44-3						35	1.544	Gabbard and Segaloff (1983)
1088	MCF-7 cells	11 -Methylestradiol-17	23637-93-6						100	2.000	Stoessel and Leclercq (1986)
1089	MCF-7 cytosol	11 -Methylestradiol-17	23637-93-6						100	2.000	Stoessel and Leclercq (1986)
1090	RUC	11 -Methylestradiol-17	23637-93-6						124	2.093	Gabbard and Segaloff (1983)
1091	RUC	7 -Methylestradiol-17 3-methyl ether	15506-01-1						5.3	0.724	Gabbard and Segaloff (1983)
1092	RUC	9 -Methylestradiol-17 3-methyl ether	51242-32-1				1				Gabbard and Segaloff (1983)
1093	RUC	11 -Methylestradiol-17 3-methyl ether	18046-75-8						5.1	0.708	Gabbard and Segaloff (1983)
1094	RUC	7 -Methylestrone	10448-96-1						68	1.833	Gabbard and Segaloff (1983)
1095	RUC	9 -Methylestrone	71563-77-4						5	0.699	Gabbard and Segaloff (1983)
1096	RUC	11 -Methylestrone	13667-06-6						47	1.672	Gabbard and Segaloff (1983)
1097	RUC	7 -Methylestrone 3-methyl ether	10449-00-0				1				Gabbard and Segaloff (1983)
1098	RUC	9 -Methylestrone 3-methyl ether	31266-41-8				1				Gabbard and Segaloff (1983)
1099	RUC	11 -Methylestrone 3-methyl ether	13667-04-4				1				Gabbard and Segaloff (1983)
1100	RUC	1-Methyl-3-ethyl-6,4'-dihydroxy-2-phenylindene							81	1.908	Anstead et al. (1989)
1101	RUC	1-Methyl-6-hydroxy-2,3-diphenylindene							12	1.079	Anstead et al. (1989)
1102	hER -FP	Methyl methacrylate	80-62-6	> 98			5000				Hashimoto et al. (2000)
1103	RUC	Methyl paraben	99-76-3	99	245	65			0.0004	-3.440	Blair et al. (2000)
1104	RUC	2-(2-Methylphenyl)-3-phenyl-6-hydroxyindene							100	2.000	Anstead et al. (1990)
1105	RUC	Methyltamoxifen	73617-95-5		0.0075				0.3	-0.900	Allen et al. (1980)
1106	RUC	<i>o,p'</i> -Methoxychlor	30667-99-3		9				0.01	-2.000	Nelson (1974)
1107	RUC	Metolachlor	51218-45-2	98.7			100				Blair et al. (2000)
1108	RUC	Mirex	2385-85-5	99			100				Blair et al. (2000)
1109	RUC	Mono- <i>m</i> -acetoxyl-1,1,2-triphenylbut-1-ene	82333-69-5						1	0.000	Jordan et al. (1986)
1110	RUC	Monohydroxymethoxychlor	28463-03-8	98	0.69	0.01			0.13	-0.890	Blair et al. (2000)
1111	RUC	Monohydroxymethoxychlor olefin	75938-34-0	98	0.39	0.08			0.23	-0.640	Blair et al. (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)††	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1112	RUC	Monohydroxytamoxifen	68392-35-8		0.012				52.1	1.800	Allen et al. (1980)
1113	RUC	Morin	480-16-0						0.00045	-3.350	Fang et al. (2001)
1114	hER	Moxestrol	34816-55-2					0.0005	43	1.633	Kuiper et al. (1997)
1115	MUC	Moxestrol	34816-55-2						4.25	0.628	Korach (1979)
1116	rER	Moxestrol	34816-55-2					0.0026	5	0.699	Kuiper et al. (1997)
1117	RUC	Moxestrol	34816-55-2		0.0065	0.0014			13.83	1.140	Blair et al. (2000)
1118	RUC	Myricetin	529-44-2						0.0018	-2.740	Fang et al. (2001)
1119	hER	Nafoxidine	1845-11-0					0.0003	44	1.643	Kuiper et al. (1997)
1120	rER	Nafoxidine	1845-11-0					0.0008	16	1.204	Kuiper et al. (1997)
1121	RUC	Nafoxidine	1845-11-0		0.125	0.055			0.72	-0.140	Blair et al. (2000)
1122	MCF-7 cells	Nafoxidine	1845-11-0						0.1	-1.000	Stoessel and Leclercq (1986)
1123	MCF-7 cytosol	Nafoxidine	1845-11-0						5	0.699	Stoessel and Leclercq (1986)
1124	GST-aERdef	Naringenin	480-41-1		4.7	0.8			0.065	-1.187	Matthews et al. (2000)
1125	GST-cERdef	Naringenin	480-41-1		39	4			0.0082	-2.086	Matthews et al. (2000)
1126	GST-hER def	Naringenin	480-41-1				100				Matthews et al. (2000)
1127	GST-mER def	Naringenin	480-41-1				100				Matthews et al. (2000)
1128	GST-rtERdef	Naringenin	480-41-1		8.7	1.3			0.039	-1.409	Matthews et al. (2000)
1129	hER	Naringenin	480-41-1						0.01	-2.000	Kuiper et al. (1998) [method a]
1130	hER	Naringenin	480-41-1						0.11	-0.959	Kuiper et al. (1998) [method a]
1131	hER	Naringenin	480-41-1		0.59				0.2	-0.699	Kuiper et al. (1998) [method b]
1132	RUC	Naringenin	480-41-1						0.0075	-2.120	Fang et al. (2001)
1133	RUC	Naringin	10236-47-2				100				Fang et al. (2001)
1134	RUC	Nerolidol	7212-44-4	97.7			1000				Blair et al. (2000)
1135	MCF-7 cytosol	4-Nitroestratrien-3-ol-17-one	5976-74-9						6	0.778	Brooks et al. (1987)
1136	MCF-7 cytosol	2-Nitroestratriene-3,17 -diol	6298-51-7						1	0.000	Brooks et al. (1987)
1137	MCF-7 cytosol	4-Nitroestratriene-3,17 -diol	6936-94-3						13	1.114	Brooks et al. (1987)
1138	MCF-7 cytosol	2-Nitroestratrien-3-ol-17-one	5976-73-8						0.1	-1.000	Brooks et al. (1987)
1139	RBC	Nitromifene	10448-84-7						0.12	-0.924	Korenman (1970)
1140	hER	cis -Nonachlor	5103-73-1				50				Klotz et al. (1996)
1141	hER	trans -Nonachlor	39765-80-5				50				Klotz et al. (1996)
1142	RUC	Nonylbenzene	1081-77-2				500				Elsby et al. (2000)
1143	hER	n -Nonylphenol	25154-52-3						0.05	-1.301	Kuiper et al. (1998) [method a]
1144	hER	n -Nonylphenol	25154-52-3		500				0.001	-3.00	Morito et al. (2001)
1145	hER	n -Nonylphenol	25154-52-3						0.09	-1.046	Kuiper et al. (1998) [method a]
1146	hER	n -Nonylphenol	25154-52-3		8				0.063	-1.20	Morito et al. (2001)
1147	MUC	n -Nonylphenol	25154-52-3	99.5			5				Shelby et al. (1996)
1148	RBC	n -Nonylphenol	25154-52-3	99.9	1.8				0.0011	-2.959	Andersen et al. (1999)
1149	hER -FP	p -Nonylphenol	104-40-5	85	3.9				0.3	-0.523	Bolger et al. (1998)
1150	hER -FP	p -Nonylphenol	104-40-5	> 93	7				0.5	-0.301	Saito et al. (2000)
1151	MCF-7 cells	p -Nonylphenol	104-40-5	Technical grade					0.026	-1.585	Nagel et al. (1997)
1152	MCF-7 cytosol	p -Nonylphenol	104-40-5		7.2	3			0.021	-1.678	Soto et al. (1995)
1153	RUC	p -Nonylphenol	104-40-5	85	4.73	0.93			0.019	-1.720	Blair et al. (2000)
1154	RUC	p -Nonylphenol	104-40-5	Technical grade	3.05	0.15			0.029	-1.530	Blair et al. (2000)
1155	RUC	p -Nonylphenol	104-40-5	85	2.9	0.8			0.031	-1.510	Blair et al. (2000)
1156	RUC	p -Nonylphenol	104-40-5	Technical grade	2.6	0.3			0.035	-1.460	Blair et al. (2000)
1157	RUC	p -Nonylphenol	104-40-5	95.6	2.4	0.3			0.037	-1.430	Blair et al. (2000)
1158	RUC	p -Nonylphenol	104-40-5	98	28	10			0.0032	-2.490	Blair et al. (2000)
1159	RUC	p -Nonylphenol	104-40-5		3				0.17	-0.770	Elsby et al. (2000)
1160	RUC	p -Nonylphenol	104-40-5	85	0.1794			0.67	0.158	-0.800	Laws et al. (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)††	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1161	RUC	<i>p</i> -Nonylphenol	104-40-5	95	8				0.0025	-2.602	Routledge et al. (1998)
1162	RUC	<i>p</i> -Nonylphenol	104-40-5					0.83	0.31	-0.504	Waller et al. (1996)
1163	RBC	Nonylphenol dodecylethoxylate					100				Andersen et al. (1999)
1164	RUC	Nordihydroguaiaretic acid	500-38-9	97	2.9	1.6			0.031	-1.510	Blair et al. (2000)
1165	hER	Norethindrone	68-22-4					0.15	0.07	-1.155	Kuiper et al. (1997)
1166	rER	Norethindrone	68-22-4					1.08	0.01	-2.000	Kuiper et al. (1997)
1167	hER	Norethynodrel	68-23-5					0.014	0.7	-0.155	Kuiper et al. (1997)
1168	rER	Norethynodrel	68-23-5					0.053	0.22	-0.658	Kuiper et al. (1997)
1169	RUC	Norethynodrel	68-23-5		0.44	0.04			0.20	-0.690	Blair et al. (2000)
1170	RUC	Norethynodrel	68-23-5	99	0.4	0.010			0.23	-0.650	Blair et al. (2000)
1171	hER	19-Nortestosterone	434-22-0					0.77	0.01	-2.000	Kuiper et al. (1997)
1172	MUC	19-Nortestosterone	434-22-0						0.1	0.950	Korach (1979)
1173	rER	19-Nortestosterone	434-22-0					0.053	0.23	-0.638	Kuiper et al. (1997)
1174	GST-aERdef	2,2',3',4,4',5,5'-Octachlorobiphenyl	35694-08-7				10				Matthews and Zacharewski (2000)
1175	GST-hER def	2,2',3',4,4',5,5'-Octachlorobiphenyl	35694-08-7				10				Matthews and Zacharewski (2000)
1176	GST-rERdef	2,2',3',4,4',5,5'-Octachlorobiphenyl	35694-08-7				10				Matthews and Zacharewski (2000)
1177	RUC	1,8-Octanediol	629-41-4	98			100				Blair et al. (2000)
1178	hER	4- <i>n</i> -Octylphenol	1806-26-4						0.07	-1.155	Kuiper et al. (1998) [method a]
1179	MCF-7 cells	4- <i>n</i> -Octylphenol	1806-26-4	Technical grade					0.072	-1.143	Nagel et al. (1997)
1180	MCF-7 cytosol	4- <i>n</i> -Octylphenol	1806-26-4		0.9				1	0.000	Dodge et al. (1996)
1181	RBC	4- <i>n</i> -Octylphenol	1806-26-4	99.4			100				Andersen et al. (1999)
1182	hER	4- <i>n</i> -Octylphenol	1806-26-4						0.02	-1.699	Kuiper et al. (1998) [method a]
1183	RUC	4- <i>n</i> -Octylphenol	1806-26-4	99	19.5	1.5			0.005	-2.340	Blair et al. (2000)
1184	GST-aERdef	4- <i>tert</i> -Octylphenol	140-66-9		3.9	1.6			0.099	-1.004	Matthews et al. (2000)
1185	GST-cERdef	4- <i>tert</i> -Octylphenol	140-66-9		0.56	0.01			0.57	-0.244	Matthews et al. (2000)
1186	GST-hER def	4- <i>tert</i> -Octylphenol	140-66-9		2.4	0.7			0.12	-0.921	Matthews et al. (2000)
1187	GST-mER def	4- <i>tert</i> -Octylphenol	140-66-9		1.6	0.1			0.17	-0.770	Matthews et al. (2000)
1188	GST-rERdef	4- <i>tert</i> -Octylphenol	140-66-9		0.11	0.02			3.2	0.505	Matthews et al. (2000)
1189	hER	4- <i>tert</i> -Octylphenol	140-66-9						0.01	-2.000	Kuiper et al. (1998) [method a]
1190	hER -FP	4- <i>tert</i> -Octylphenol	140-66-9	97	7.5				0.2	-0.699	Bolger et al. (1998)
1191	hER	4- <i>tert</i> -Octylphenol	140-66-9						0.03	-1.523	Kuiper et al. (1998) [method a]
1192	RUC	4- <i>tert</i> -Octylphenol	140-66-9					1.32	0.197	-0.706	Waller et al. (1996)
1193	RUC	4- <i>tert</i> -Octylphenol	140-66-9	97	6.0	1.10			0.015	-1.820	Blair et al. (2000)
1194	RUC	4- <i>tert</i> -Octylphenol	140-66-9	97	0.2085			0.78	0.51	-0.291	Laws et al. (2000)
1195	GST-aERdef	2,2',3',3',6-Pentachlorobiphenyl	52663-60-2				10				Matthews and Zacharewski (2000)
1196	GST-hER def	2,2',3',3',6-Pentachlorobiphenyl	52663-60-2				10				Matthews and Zacharewski (2000)
1197	GST-rERdef	2,2',3',3',6-Pentachlorobiphenyl	52663-60-2				10				Matthews and Zacharewski (2000)
1198	GST-aERdef	2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8				10				Matthews and Zacharewski (2000)
1199	GST-hER def	2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8				10				Matthews and Zacharewski (2000)
1200	GST-rERdef	2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8				10				Matthews and Zacharewski (2000)
1201	GST-aERdef	2,2',3,4',6-Pentachlorobiphenyl	68194-05-8				10				Matthews and Zacharewski (2000)
1202	GST-hER def	2,2',3,4',6-Pentachlorobiphenyl	68194-05-8				10				Matthews and Zacharewski (2000)
1203	GST-rERdef	2,2',3,4',6-Pentachlorobiphenyl	68194-05-8		10				0.031	-1.509	Matthews and Zacharewski (2000)
1204	GST-aERdef	2,2',3,5',6-Pentachlorobiphenyl	38379-99-6				10				Matthews and Zacharewski (2000)
1205	GST-hER def	2,2',3,5',6-Pentachlorobiphenyl	38379-99-6				10				Matthews and Zacharewski (2000)
1206	GST-rERdef	2,2',3,5',6-Pentachlorobiphenyl	38379-99-6				10				Matthews and Zacharewski (2000)
1207	GST-aERdef	2,2',4,4',5-Pentachlorobiphenyl	38380-01-7				10				Matthews and Zacharewski (2000)
1208	GST-hER def	2,2',4,4',5-Pentachlorobiphenyl	38380-01-7				10				Matthews and Zacharewski (2000)
1209	GST-rERdef	2,2',4,4',5-Pentachlorobiphenyl	38380-01-7				10				Matthews and Zacharewski (2000)

Data Sorted by Substance and Assay

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1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1210	GST-aERdef	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2				10				Matthews and Zacharewski (2000)
1211	GST-hER def	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2				10				Matthews and Zacharewski (2000)
1212	GST-rtERdef	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2				10				Matthews and Zacharewski (2000)
1213	GST-aERdef	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8		10				0.025	-1.602	Matthews and Zacharewski (2000)
1214	GST-hER def	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8		10				0.024	-1.620	Matthews and Zacharewski (2000)
1215	GST-rtERdef	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8		1.3	0.6			0.24	-0.623	Matthews and Zacharewski (2000)
1216	MUC	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8	> 98	1.7				0.88	-0.056	Fielden et al. (1997)
1217	GST-aERdef	2,3,3',5,6'-Pentachlorobiphenyl	74472-36-9				10				Matthews and Zacharewski (2000)
1218	GST-hER def	2,3,3',5,6'-Pentachlorobiphenyl	74472-36-9				10				Matthews and Zacharewski (2000)
1219	GST-rtERdef	2,3,3',5,6'-Pentachlorobiphenyl	74472-36-9				10				Matthews and Zacharewski (2000)
1220	GST-aERdef	2,3,4,4',6'-Pentachlorobiphenyl	74472-38-1				10				Matthews and Zacharewski (2000)
1221	GST-hER def	2,3,4,4',6'-Pentachlorobiphenyl	74472-38-1				10				Matthews and Zacharewski (2000)
1222	GST-rtERdef	2,3,4,4',6'-Pentachlorobiphenyl	74472-38-1		10				0.031	-1.509	Matthews and Zacharewski (2000)
1223	GST-aERdef	3,3',4,4',5'-Pentachlorobiphenyl	57465-28-8				10				Matthews and Zacharewski (2000)
1224	GST-hER def	3,3',4,4',5'-Pentachlorobiphenyl	57465-28-8				10				Matthews and Zacharewski (2000)
1225	GST-rtERdef	3,3',4,4',5'-Pentachlorobiphenyl	57465-28-8				10				Matthews and Zacharewski (2000)
1226	hER	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
1227	hER	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	> 98					0.13	-0.886	Kuiper et al. (1998) [method a]
1228	MUC	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	> 98	6.3				0.033	-1.480	Connor et al. (1997)
1229	RUC	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	> 98	40				0.036	-1.440	Connor et al. (1997)
1230	hER	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	> 98					0.3	-0.523	Kuiper et al. (1998) [method a]
1231	hER	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	> 98					0.2	-0.699	Kuiper et al. (1998) [method a]
1232	MUC	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	> 98	4.8				0.044	-1.360	Connor et al. (1997)
1233	RUC	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	> 98	12				0.12	-0.920	Connor et al. (1997)
1234	hER	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	> 98					0.09	-1.046	Kuiper et al. (1998) [method a]
1235	hER	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	> 98					0.03	-1.523	Kuiper et al. (1998) [method a]
1236	MUC	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	> 98	16				0.013	-1.890	Connor et al. (1997)
1237	RUC	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	> 98	10				0.14	-0.850	Connor et al. (1997)
1238	MUC	2,2',4,6,6'-Pentachloro-4-biphenylol		> 98	0.07				21.43	1.331	Fielden et al. (1997)
1239	MCF-7 cytosol	2',3,3',4,4'-Pentachloro-2-biphenylol	150975-80-7	> 95	5.7	0.2			0.004	-2.398	Kramer et al. (1997)
1240	hER	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3	> 98					0.01	-2.000	Kuiper et al. (1998) [method a]
1241	hER	2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	> 98					0.11	-0.959	Kuiper et al. (1998) [method a]
1242	hER	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3	> 98			10				Kuiper et al. (1998) [method a]
1243	hER	2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	> 98					0.11	-0.959	Kuiper et al. (1998) [method a]
1244	MUC	2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	> 98	2.9				0.072	-1.140	Connor et al. (1997)
1245	RUC	2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	> 98	17				0.082	-1.090	Connor et al. (1997)
1246	hER	2,3,3',4',5'-Pentachloro-4-biphenylol	152969-11-4	> 98					0.03	-1.523	Kuiper et al. (1998) [method a]
1247	hER	2,3,3',4',5'-Pentachloro-4-biphenylol	152969-11-4	> 98					0.11	-1.699	Kuiper et al. (1998) [method a]
1248	MCF-7 cytosol	2,3,3',4',5'-Pentachloro-4-biphenylol	152969-11-4	> 95	3.3	0.2			1	0.000	Kramer et al. (1997)
1249	MCF-7 cytosol	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3	> 95	4.2				0.1	-1.000	Kramer et al. (1997)
1250	MCF-7 cytosol	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3	> 95	4.2				0.1	-1.000	Kramer et al. (1997)
1251	GST-aERdef	2,3,3',4',5'-Pentachloro-4-biphenylol	152969-11-4				10				Matthews and Zacharewski (2000)
1252	GST-hER def	2,3,3',4',5'-Pentachloro-4-biphenylol	152969-11-4				10				Matthews and Zacharewski (2000)
1253	GST-rtERdef	2,3,3',4',5'-Pentachloro-4-biphenylol	152969-11-4				10				Matthews and Zacharewski (2000)
1254	GST-rtERdef	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3				10				Matthews and Zacharewski (2000)
1255	hER	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	> 98					0.13	-0.886	Kuiper et al. (1998) [method a]
1256	hER	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	> 98					0.12	-0.921	Kuiper et al. (1998) [method a]
1257	MUC	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	> 98			1000				Connor et al. (1997)
1258	RUC	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	> 98	35				0.041	-1.390	Connor et al. (1997)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1259	hER	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	> 98					0.06	-1.222	Kuiper et al. (1998) [method a]
1260	hER	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	> 98					0.04	-1.398	Kuiper et al. (1998) [method a]
1261	MUC	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	> 98	6.7				0.031	-1.520	Connor et al. (1997)
1262	RUC	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	> 98	21				0.068	-1.170	Connor et al. (1997)
1263	MCF-7 cytosol	2',3,4,4',5-Pentachloro-3-biphenylol	150975-81-8	> 95	3	1.2			2	0.301	Kramer et al. (1997)
1264	MCF-7 cytosol	2',3,4,4',5-Pentachloro-3-biphenylol	170946-11-9	> 95	3.3	0.2			1	0.000	Kramer et al. (1997)
1265	MCF-7 cytosol	2',3,4',5,5'-Pentachloro-4-biphenylol	149589-56-0						0.8	-0.969	Kramer et al. (1997)
1266	MCF-7 cytosol	3,3',4',5,5'-Pentachloro-4-biphenylol	130689-92-8	> 95	5.1	1.8			0.02	-1.699	Kramer et al. (1997)
1267	RUC	Pentolame	150748-24-6		20				0.04	-1.398	Jaimez et al. (2000)
1268	hER -FP	Permethrin	52645-53-1	> 93			10				Saito et al. (2000)
1269	MCF-7 cells	Phenanthrene	85-01-8				5				Arcaro et al. (1999)
1270	RUC	4-Phenethylphenol	6335-83-7		44	6			0.002	-2.690	Blair et al. (2000)
1271	MCF-7 cells	Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis-	107144-81-0						26	1.415	Stoessel and Leclercq (1986)
1272	MCF-7 cytosol	Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis-	107144-81-0						20	1.301	Stoessel and Leclercq (1986)
1273	MCF-7 cells	Phenol, 4-[7-(2 Dimethylamino)- ethoxy]-11-ethylidibenzo- [b,f]thiepin-10-yl]-	85850-74-4						0.3	-0.523	Stoessel and Leclercq (1986)
1274	MCF-7 cytosol	Phenol, 4-[7-(2 Dimethylamino)- ethoxy]-11-ethylidibenzo- [b,f]thiepin-10-yl]-	85850-74-4						63	1.799	Stoessel and Leclercq (1986)
1275	RUC	Phenol, 4-[7-(2 Dimethylamino)- ethoxy]-11-ethylidibenzo- [b,f]thiepin-10-yl]-	85850-74-4						63	1.799	Acton et al. (1983)
1276	MCF-7 cells	Phenol, 4-[3-(2 dimethylamino)- ethoxy]-11-ethylidibenzo- [b,f]thioctin-12-yl)-	85850-81-3						2.5	0.398	Stoessel and Leclercq (1986)
1277	MCF-7 cytosol	Phenol, 4-[3-(2 dimethylamino)ethoxy]-11-ethylidibenzo[b,f]thioctin-12-yl)	85850-81-3						50	1.699	Stoessel and Leclercq (1986)
1278	RUC	Phenol, 4-[3-(2 dimethylamino)- ethoxy]-11-ethylidibenzo- [b,f]thioctin-12-yl)-	85850-81-3						52	1.716	Acton et al. (1983)
1279	MCF-7 cells	Phenol, 4-[2-(2 dimethylamino)- ethoxy]-6-ethyl-11,12-dihydro- dibenzo[a,e]-cycloocten-5-yl]-	85850-75-5						1.3	0.114	Stoessel and Leclercq (1986)
1280	MCF-7 cytosol	Phenol, 4-[2-(2 dimethylamino)- ethoxy]-6-ethyl-11,12-dihydro- dibenzo[a,e]-cycloocten-5-yl]-	85850-75-5						50	1.699	Stoessel and Leclercq (1986)
1281	RUC	Phenol, 4-[2-(2 dimethylamino)- ethoxy]-6-ethyl-11,12-dihydro- dibenzo[a,e]-cycloocten-5-yl]-	85850-75-5						50	1.699	Acton et al. (1983)
1282	MCF-7 cells	Phenol, 3-[2- dimethylamino- ethoxy]- -10 ethyl- 4-hydroxy- phenyl dibenzo- [b,f]oxepin	85850-80-2						0.1	-1.000	Stoessel and Leclercq (1986)
1283	MCF-7 cytosol	Phenol, 3-[2- dimethylamino- ethoxy]- -10 ethyl- 4-hydroxy- phenyl dibenzo- [b,f]oxepin	85850-80-2						6	0.778	Stoessel and Leclercq (1986)
1284	RUC	Phenol, 3-[2- dimethylamino- ethoxy]- -10 ethyl- 4-hydroxy- phenyl dibenzo- [b,f]oxepin	85850-80-2						6.1	0.785	Acton et al. (1983)
1285	MCF-7 cells	Phenol, 4-[1-[4-[2-(dimethyl- amino) ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (E)-	96474-35-0						0.4	-0.398	Stoessel and Leclercq (1986)
1286	MCF-7 cytosol	Phenol, 4-[1-[4-[2-(dimethyl- amino) ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (E)-	96474-35-0						100	2.000	Stoessel and Leclercq (1986)
1287	MCF-7 cells	Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9						2	0.301	Stoessel and Leclercq (1986)
1288	MCF-7 cytosol	Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9						15	1.176	Stoessel and Leclercq (1986)
1289	MCF-7 cells	Phenol, 4-(1Z)-1,2-diphenyl-1-butenyl)-	69967-80-2						0.4	-0.398	Stoessel and Leclercq (1986)
1290	MCF-7 cytosol	Phenol, 4-(1Z)-1,2-diphenyl-1-butenyl)-	69967-80-2						1	0.000	Stoessel and Leclercq (1986)
1291	MCF-7 cells	Phenol, 4-[2-Nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy]-phenyl]ethenyl]phenyl, (E)-	107144-84-3						2.1	0.322	Stoessel and Leclercq (1986)
1292	MCF-7 cytosol	Phenol, 4-[2-Nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy]-phenyl]ethenyl]phenyl, (E)-	107144-84-3						100	2.000	Stoessel and Leclercq (1986)
1293	MCF-7 cells	Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-	91221-46-4						2	0.301	Stoessel and Leclercq (1986)
1294	MCF-7 cytosol	Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-	91221-46-4						100	2.000	Stoessel and Leclercq (1986)
1295	RUC	Phenolphthalein	77-09-8		6.73	0.018			0.013	-1.870	Blair et al. (2000)
1296	RUC	Phenolphthalin	81-90-3	99	425	75			0.0002	-3.670	Blair et al. (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1297	RUC	Phenol Red	143-74-8	95	160	60			0.001	-3.250	Blair et al. (2000)
1298	hER -FP	<i>d</i> -Phenothrin	26002-80-2	> 93			10				Saito et al. (2000)
1299	RUC	2-Phenyl-3-(2-fluoro-4-hydroxyphenyl)-6-hydroxyindene							9.6	0.982	Anstead et al. (1990)
1300	RUC	2-Phenyl-3-(2-fluorophenyl)-6-hydroxyindene							12	1.079	Anstead et al. (1990)
1301	RUC	3-Phenyl-4'-hydroxy-2-phenylindene							0.017	-1.770	Anstead et al. (1989)
1302	RUC	3-Phenyl-4'-hydroxy-2-phenylindene							0.36	-0.444	Anstead et al. (1989)
1303	RUC	3-Phenyl-6-hydroxy-2-phenylindene							8.9	0.949	Anstead et al. (1989)
1304	RUC	2-Phenyl-3-(2-methylphenyl)-6-hydroxyindene							11	1.041	Anstead et al. (1990)
1305	RUC	2-Phenyl-3-(4-methylphenyl)-6-hydroxyindene							1.7	0.230	Anstead et al. (1990)
1306	RUC	2-Phenylphenol	90-43-7	99			100				Blair et al. (2000)
1307	RUC	3-Phenylphenol	580-51-8	90	245	45			0.0004	-3.440	Blair et al. (2000)
1308	MUC	4-Phenylphenol	92-69-3	> 98			5				Korach et al. (1988)
1309	RUC	4-Phenylphenol	92-69-3	90	98	52			0.001	-3.040	Blair et al. (2000)
1310	hER	Phloretin	60-82-2						0.2	-0.699	Kuiper et al. (1998) [method a]
1311	hER	Phloretin	60-82-2						0.7	-0.155	Kuiper et al. (1998) [method a]
1312	RUC	Phloretin	60-82-2						0.069	-1.160	Fang et al. (2001)
1313	hER -FP	Prallethrin	23031-36-9	> 93			10				Saito et al. (2000)
1314	hER	Progesterone	57-83-0				100				Kuiper et al. (1997)
1315	hER	Progesterone	57-83-0				10				Kuiper et al. (1998) [method a]
1316	hER	Progesterone	57-83-0				10				Kuiper et al. (1998) [method a]
1317	MUC	Progesterone	57-83-0				10				Korach (1979)
1318	rER	Progesterone	57-83-0				100				Kuiper et al. (1997)
1319	RUC	Progesterone	57-83-0	99			1000				Blair et al. (2000)
1320	RUC	Progesterone	57-83-0				2667				Laws et al. (2000)
1321	RUC	Progesterone	57-83-0					1000	0.0003	-3.523	Waller et al. (1996)
1322	RUC	Prolame	99876-41-2		7				0.11	-0.959	Jaimez et al. (2000)
1323	RUC	Promegestone	34184-77-5	98			2667				Laws et al. (2000)
1324	MUC	Promegestone	34184-77-5				10				Korach (1979)
1325	RUC	Promegestone	34184-77-5					1.18	0.22	-0.658	Waller et al. (1996)
1326	RUC	Prometon	1610-18-0				1000				Blair et al. (2000)
1327	hER -FP	Propazine	139-40-2	99.9			2000				Hanioka et al. (1999)
1328	RUC	Propyl paraben	94-13-3	99	150	10			0.0006	-3.220	Blair et al. (2000)
1329	hER	Propylpyrazoletriol							49	1.690	Kraichely et al. (2000)
1330	hER	Propylpyrazoletriol							0.12	-0.921	Kraichely et al. (2000)
1331	RUC	Prunetin	552-59-0						0.0018	-2.740	Fang et al. (2001)
1332	MUC	Pseudodiethylstilbestrol	39011-86-4		0.0011	0.0002			91	1.960	Korach et al. (1979)
1333	MUC	Pseudodiethylstilbestrol	39011-86-4		1.1	0.2			145.5	2.160	Korach et al. (1985)
1334	MCF-7 cells	Pyrene	129-00-0				5				Arcaro et al. (1999)
1335	MCF-7 cells	Pyrolidone, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (E)	77413-87-7						0.07	-1.155	Stoessel and Leclercq (1986)
1336	MCF-7 cytosol	Pyrolidone, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (E)	77413-87-7						11	1.041	Stoessel and Leclercq (1986)
1337	hER	Quercetin	117-39-5						0.01	-2.000	Kuiper et al. (1998) [method a]
1338	hER	Quercetin	117-39-5						0.04	-1.398	Kuiper et al. (1998) [method a]
1339	MCF-7 cytosol	Quercetin	117-39-5				25				Miodini et al. (1999)
1340	RUC	Quercetin	117-39-5				100				Fang et al. (2001)
1341	GST-aERdef	Quercetin	117-39-5		19	2			0.016	-1.796	Matthews et al. (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1342	GST-cERdef	Quercetin	117-39-5		82	22			0.0039	-2.409	Matthews et al. (2000)
1343	GST-hER def	Quercetin	117-39-5				100			n.a	Matthews et al. (2000)
1344	GST-mER def	Quercetin	117-39-5				100				Matthews et al. (2000)
1345	GST-rERdef	Quercetin	117-39-5		8	2			0.042	-1.377	Matthews et al. (2000)
1346	MCF-7 cells	6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1						22	1.342	Stoessel and Leclercq (1986)
1347	MCF-7 cytosol	6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1						33	1.519	Stoessel and Leclercq (1986)
1348	MCF-7 cells	7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2						3	0.477	Stoessel and Leclercq (1986)
1349	MCF-7 cytosol	7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2						9	0.954	Stoessel and Leclercq (1986)
1350	hER	Raloxifene	84449-90-1						69	1.839	Kuiper et al. (1998) [method a]
1351	hER	Raloxifene	84449-90-1		0.0018				51.7	1.700	Kuiper et al. (1998) [method b]
1352	hER	Raloxifene	84449-90-1						16	1.204	Kuiper et al. (1998) [method a]
1353	hER	Raloxifene	84449-90-1		0.0041				25.9	1.410	Kuiper et al. (1998) [method b]
1354	hER	Raloxifene hydrochloride	82640-04-8		0.0008				62.5	1.796	Arcaro et al. (2000)
1355	RUC	Resveratrol	501-36-0		0.001				300	2.477	Ashby et al. (1999)
1356	RUC	Rutin	153-18-4						0.000082	-4.090	Fang et al. (2001)
1357	GST-aERdef	Simazine	122-34-9				100				Matthews et al. (2000)
1358	GST-cERdef	Simazine	122-34-9				100				Matthews et al. (2000)
1359	GST-hER def	Simazine	122-34-9				100				Matthews et al. (2000)
1360	GST-mER def	Simazine	122-34-9				100				Matthews et al. (2000)
1361	GST-rERdef	Simazine	122-34-9				100				Matthews et al. (2000)
1362	hER -FP	Simazine	122-34-9	99.4			2000				Hanioka et al. (1999)
1363	RUC	Simazine	122-34-9	99			33.3				Blair et al. (2000)
1364	GST-aERdef	-Sitosterol	83-46-5				100				Matthews et al. (2000)
1365	GST-cERdef	-Sitosterol	83-46-5				100				Matthews et al. (2000)
1366	GST-hER def	-Sitosterol	83-46-5				100			n.a	Matthews et al. (2000)
1367	GST-mER def	-Sitosterol	83-46-5				100				Matthews et al. (2000)
1368	GST-rERdef	-Sitosterol	83-46-5				100				Matthews et al. (2000)
1369	hER	-Sitosterol	83-46-5				100				Kuiper et al. (1997)
1370	rER	-Sitosterol	83-46-5				100				Kuiper et al. (1997)
1371	RUC	-Sitosterol	83-46-5				1000				Fang et al. (2001)
1372	RUC	4,4'-Stilbenediol	659-22-3		0.32	0.09			0.281	-0.550	Blair et al. (2000)
1373	RUC	4-Stilbenol	3839-46-1				100				Blair et al. (2000)
1374	RUC	Suberic acid	505-48-6	99			100				Blair et al. (2000)
1375	GST-aERdef	Tamoxifen	10540-29-1		0.03	0.003			10	1.000	Matthews et al. (2000)
1376	GST-cERdef	Tamoxifen	10540-29-1		0.021	0.001			16	1.204	Matthews et al. (2000)
1377	GST-hER def	Tamoxifen	10540-29-1		0.028	0.004			11	1.041	Matthews et al. (2000)
1378	GST-mER def	Tamoxifen	10540-29-1		0.026	0.001			10	1.000	Matthews et al. (2000)
1379	GST-rERdef	Tamoxifen	10540-29-1		0.013	0.001			25	1.398	Matthews et al. (2000)
1380	hER	Tamoxifen	10540-29-1					0.0034	7	0.845	Kuiper et al. (1997)
1381	hER	Tamoxifen	10540-29-1						4	0.602	Kuiper et al. (1998) [method a]
1382	hER	Tamoxifen	10540-29-1		0.17				2.94	0.47	Morito et al. (2001)
1383	hER -FP	Tamoxifen	10540-29-1		0.423				3.1	0.491	Bolger et al. (1998)
1384	hER -FP	Tamoxifen	10540-29-1		0.189				2.96	0.471	Parker et al. (2000)
1385	hER	Tamoxifen	10540-29-1						3	0.477	Kuiper et al. (1998) [method a]
1386	hER	Tamoxifen	10540-29-1		0.3				1.67	0.22	Morito et al. (2001)
1387	MUC	Tamoxifen	10540-29-1	99	0.0275				6.55	0.816	Shelby et al. (1996)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)††	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1388	RBC	Tamoxifen	10540-29-1	96	0.12				0.017	-1.770	Andersen et al. (1999)
1389	rER	Tamoxifen	10540-29-1					0.0025	6	0.778	Kuiper et al. (1997)
1390	RUC	Tamoxifen	10540-29-1						5.9	0.771	Acton et al. (1983)
1391	RUC	Tamoxifen	10540-29-1		6				0.13	-0.523	Allen et al. (1980)
1392	RUC	Tamoxifen	10540-29-1		0.063				0.3	-0.523	Liu et al. (1994)
1393	RUC	Tamoxifen	10540-29-1						6	0.778	Qian and Abul-Hajj (1990)
1394	MCF-7 cells	Tamoxifen	10540-29-1						0.06	-1.222	Stoessel and Leclercq (1986)
1395	MCF-7 cytosol	Tamoxifen	10540-29-1						1	0.000	Stoessel and Leclercq (1986)
1396	RUC	Tamoxifen citrate	54965-24-1		0.0555	0.0005			1.62	0.210	Blair et al. (2000)
1397	RUC	Taxifolin	480-18-2				100				Fang et al. (2001)
1398	hER	Testosterone	58-22-0				100				Kuiper et al. (1997)
1399	hER	Testosterone	58-22-0				10				Kuiper et al. (1998) [method a]
1400	hER -FP	Testosterone	58-22-0		35	0.5			0.04	-1.398	Nikov et al. (2000)
1401	hER	Testosterone	58-22-0				10				Kuiper et al. (1998) [method a]
1402	MUC	Testosterone	58-22-0				10				Korach (1979)
1403	RBC	Testosterone	58-22-0	100			100				Andersen et al. (1999)
1404	rER	Testosterone	58-22-0				100				Kuiper et al. (1997)
1405	RUC	Testosterone	58-22-0	98			1000				Blair et al. (2000)
1406	RUC	Testosterone	58-22-0					28.97	0.01	-2.000	Waller et al. (1996)
1407	GST-aERdef	2,2',3,3'-Tetrachlorobiphenyl	3844-93-8				10				Matthews and Zacharewski (2000)
1408	GST-hER def	2,2',3,3'-Tetrachlorobiphenyl	3844-93-8				10				Matthews and Zacharewski (2000)
1409	GST-rtERdef	2,2',3,3'-Tetrachlorobiphenyl	3844-93-8				10				Matthews and Zacharewski (2000)
1410	GST-aERdef	2,2',3,4'-Tetrachlorobiphenyl	52663-59-9				10				Matthews and Zacharewski (2000)
1411	GST-hER def	2,2',3,4'-Tetrachlorobiphenyl	52663-59-9				10				Matthews and Zacharewski (2000)
1412	GST-rtERdef	2,2',3,4'-Tetrachlorobiphenyl	52663-59-9		10				0.024	-1.620	Matthews and Zacharewski (2000)
1413	GST-aERdef	2,2',3,6'-Tetrachlorobiphenyl	41464-47-5				10				Matthews and Zacharewski (2000)
1414	GST-hER def	2,2',3,6'-Tetrachlorobiphenyl	41464-47-5				10				Matthews and Zacharewski (2000)
1415	GST-rtERdef	2,2',3,6'-Tetrachlorobiphenyl	41464-47-5		10				0.031	-1.509	Matthews and Zacharewski (2000)
1416	RUC	2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	98.4			100				Blair et al. (2000)
1417	GST-aERdef	2,2',4,4'-Tetrachlorobiphenyl	2437-79-8				10				Matthews and Zacharewski (2000)
1418	GST-hER def	2,2',4,4'-Tetrachlorobiphenyl	2437-79-8				10			n.a	Matthews and Zacharewski (2000)
1419	GST-rtERdef	2,2',4,4'-Tetrachlorobiphenyl	2437-79-8		10				0.031	-1.509	Matthews and Zacharewski (2000)
1420	GST-aERdef	2,2',4,5'-Tetrachlorobiphenyl	41464-40-8				10				Matthews and Zacharewski (2000)
1421	GST-hER def	2,2',4,5'-Tetrachlorobiphenyl	41464-40-8				10				Matthews and Zacharewski (2000)
1422	GST-rtERdef	2,2',4,5'-Tetrachlorobiphenyl	41464-40-8				10				Matthews and Zacharewski (2000)
1423	GST-aERdef	2,2',4,6'-Tetrachlorobiphenyl	68194-04-7				10				Matthews and Zacharewski (2000)
1424	GST-hER def	2,2',4,6'-Tetrachlorobiphenyl	68194-04-7				10				Matthews and Zacharewski (2000)
1425	GST-rtERdef	2,2',4,6'-Tetrachlorobiphenyl	68194-04-7		10				0.031	-1.509	Matthews and Zacharewski (2000)
1426	hER	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	≥ 99%			50				Vakharia and Gierthy (2000)
1427	GST-aERdef	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5				10				Matthews and Zacharewski (2000)
1428	GST-hER def	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5				10			n.a	Matthews and Zacharewski (2000)
1429	GST-rtERdef	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5		10				0.031	-1.509	Matthews and Zacharewski (2000)
1430	hER	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	≥ 99			100				Arcaro et al. (1999)
1431	hER	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	≥ 99%			50				Vakharia and Gierthy (2000)
1432	hER	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	≥ 99			100				Arcaro et al. (1999)
1433	GST-aERdef	2,3,3',5'-Tetrachlorobiphenyl	41464-49-7				10				Matthews and Zacharewski (2000)
1434	GST-hER def	2,3,3',5'-Tetrachlorobiphenyl	41464-49-7				10				Matthews and Zacharewski (2000)
1435	GST-rtERdef	2,3,3',5'-Tetrachlorobiphenyl	41464-49-7				10				Matthews and Zacharewski (2000)
1436	GST-aERdef	2,3,4,4'-Tetrachlorobiphenyl	33025-41-1				10				Matthews and Zacharewski (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1437	GST-hER def	2,3,4,4'-Tetrachlorobiphenyl	33025-41-1				10				Matthews and Zacharewski (2000)
1438	GST-rtERdef	2,3,4,4'-Tetrachlorobiphenyl	33025-41-1				10				Matthews and Zacharewski (2000)
1439	GST-aERdef	2,3',4',5'-Tetrachlorobiphenyl	32598-11-1				10				Matthews and Zacharewski (2000)
1440	GST-hER def	2,3',4',5'-Tetrachlorobiphenyl	32598-11-1				10				Matthews and Zacharewski (2000)
1441	GST-rtERdef	2,3',4',5'-Tetrachlorobiphenyl	32598-11-1				10				Matthews and Zacharewski (2000)
1442	GST-aERdef	2,3',4',5'-Tetrachlorobiphenyl	73575-52-7				10				Matthews and Zacharewski (2000)
1443	GST-hER def	2,3',4',5'-Tetrachlorobiphenyl	73575-52-7				10				Matthews and Zacharewski (2000)
1444	GST-rtERdef	2,3',4',5'-Tetrachlorobiphenyl	73575-52-7				10				Matthews and Zacharewski (2000)
1445	GST-aERdef	2,4,4',5'-Tetrachlorobiphenyl	32690-93-0				10				Matthews and Zacharewski (2000)
1446	GST-hER def	2,4,4',5'-Tetrachlorobiphenyl	32690-93-0				10				Matthews and Zacharewski (2000)
1447	GST-rtERdef	2,4,4',5'-Tetrachlorobiphenyl	32690-93-0				10				Matthews and Zacharewski (2000)
1448	hER	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	≥ 99%			50				Vakharia and Gierthy (2000)
1449	GST-aERdef	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3				10				Matthews and Zacharewski (2000)
1450	GST-hER def	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3				10				Matthews and Zacharewski (2000)
1451	GST-rtERdef	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3				10				Matthews and Zacharewski (2000)
1452	RUC	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	99			300				Blair et al. (2000)
1453	GST-aERdef	3,3',4,5'-Tetrachlorobiphenyl	70362-49-1				10				Matthews and Zacharewski (2000)
1454	GST-hER def	3,3',4,5'-Tetrachlorobiphenyl	70362-49-1				10				Matthews and Zacharewski (2000)
1455	GST-rtERdef	3,3',4,5'-Tetrachlorobiphenyl	70362-49-1				10				Matthews and Zacharewski (2000)
1456	MUC	2',3',5',6'-Tetrachloro-4,4'-biphenyldiol	100702-98-5	> 98	5.0				0.020	-1.699	Korach et al. (1988)
1457	MUC	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	> 98	1.354				0.074	-1.130	Korach et al. (1988)
1458	MUC	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3					1.95	0.13	-0.880	Waller et al. (1996)
1459	MCF-7 cytosol	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	> 95	3.7	0.3			0.4	-0.398	Kramer et al. (1997)
1460	RUC	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	95	160	10			0.001	-3.250	Blair et al. (2000)
1461	hER	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	> 98					0.3	-0.523	Kuiper et al. (1998) [method a]
1462	hER	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	> 98					0.5	-0.301	Kuiper et al. (1998) [method a]
1463	MUC	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	> 98	12				0.018	-1.740	Connor et al. (1997)
1464	RUC	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	> 98	2700				0.00053	-3.275	Connor et al. (1997)
1465	hER	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.2				0.3	-0.520	Arcaro et al. (1999)
1466	hER	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.2				0.3	-0.520	Arcaro et al. (1999)
1467	hER	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		1.995				0.25	-0.600	Arcaro et al. (1999)
1468	hER	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		1.995				0.25	-0.600	Arcaro et al. (1999)
1469	GST-aERdef	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.5	0.2			0.50	-0.301	Matthews and Zacharewski (2000)
1470	GST-hER def	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.5	0.02			0.48	-0.319	Matthews and Zacharewski (2000)
1471	GST-rtERdef	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.3	0.1			1.03	0.013	Matthews and Zacharewski (2000)
1472	hER	2,2',6,6'-tetrachloro-4-biphenylol	219952-18-8	95 - 99	0.5				0.20	-0.700	Vakharia and Gierthy (2000)
1473	MCF-7 cytosol	2',3',4',5'-Tetrachloro-3-biphenylol	67651-37-0	> 95	4.3	0.7			0.1	-1.000	Kramer et al. (1997)
1474	GST-aERdef	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		0.5	0.2			0.50	-0.301	Matthews and Zacharewski (2000)
1475	hER	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 98					3.4	0.531	Kuiper et al. (1998) [method a]
1476	hER	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 98					7.2	0.857	Kuiper et al. (1998) [method a]
1477	MUC	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 98	0.0950				1.05	0.021	Korach et al. (1988)
1478	MUC	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 98	0.990				1.11	0.046	Ramamoorthy et al. (1997b)
1479	MUC	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7					0.0452	5.75	0.760	Waller et al. (1996)
1480	MCF-7 cytosol	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 95	2.8	0.6			3.2	0.505	Kramer et al. (1997)
1481	GST-hER def	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		0.1	0.02			2.4	0.380	Matthews and Zacharewski (2000)
1482	GST-rtERdef	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		0.27	0.02			1.15	0.061	Matthews and Zacharewski (2000)
1483	RUC	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	95	0.395	0.015			0.23	-0.640	Blair et al. (2000)
1484	hER	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	> 98					0.18	-0.745	Kuiper et al. (1998) [method a]
1485	hER	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	> 98					0.23	-0.638	Kuiper et al. (1998) [method a]

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)†‡	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1486	MUC	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	> 98			1000				Connor et al. (1997)
1487	RUC	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	> 98			1000				Connor et al. (1997)
1488	MCF-7 cells	2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin	1746-01-6				5				Arcaro et al. (1999)
1489	hER	Tetrahydrochrysene	104460-72-2						3	0.477	Meyers et al. (1999)
1490	hER	Tetrahydrochrysene	104460-72-2						6.5	0.813	Meyers et al. (1999)
1491	hER	(<i>rac</i>)-Tetrahydrochrysene						0.0036	25	1.398	Sun et al. (1999)
1492	hER	(<i>rac</i>)-Tetrahydrochrysene						0.013	2.5	0.398	Sun et al. (1999)
1493	hER	(<i>R,R</i>)-Tetrahydrochrysene						0.009	3.6	0.556	Sun et al. (1999)
1494	hER	(<i>R,R</i>)-Tetrahydrochrysene						0.0036	25	1.398	Sun et al. (1999)
1495	hER	(<i>S,S</i>)-Tetrahydrochrysene						0.039	0.83	-0.081	Sun et al. (1999)
1496	hER	(<i>S,S</i>)-Tetrahydrochrysene						0.07	1.3	0.114	Sun et al. (1999)
1497	RUC	2,2',4,4'-Tetrahydroxybenzil	5394-98-9		0.43	0			0.209	-0.680	Blair et al. (2000)
1498	MCF-7 cells	Tetramethylhexestrol	74385-27-6						2	0.301	Stoessel and Leclercq (1986)
1499	MCF-7 cytosol	Tetramethylhexestrol	74385-27-6						1.5	0.176	Stoessel and Leclercq (1986)
1500	RUC	Thalidomide	50-35-1	99			1000				Blair et al. (2000)
1501	RUC	Toremifene citrate	89778-27-8		0.065	0.005			1.38	0.140	Blair et al. (2000)
1502	RUC	Tosyl Nonylphenol (mixed branched isomers)			80				0.006	-2.204	Elsby et al. (2000)
1503	hER	Toxaphene	8001-35-2	95 - 99			10				Arcaro et al. (2000)
1504	MCF-7 cytosol	Toxaphene	8001-35-2	Technical grade	470	38			0.00032	-3.495	Soto et al. (1995)
1505	MUC	Toxaphene	8001-35-2	Technical grade			10				Ramamoorthy et al. (1997a)
1506	hER	Triaryl-pyrazole						0.00054	60	1.778	Sun et al. (1999)
1507	hER	Triaryl-pyrazole						0.0051	18	1.255	Sun et al. (1999)
1508	GST-aERdef	2,2',5-Trichlorobiphenyl	37680-65-2				10				Matthews and Zacharewski (2000)
1509	GST-hER def	2,2',5-Trichlorobiphenyl	37680-65-2				10				Matthews and Zacharewski (2000)
1510	GST-rtERdef	2,2',5-Trichlorobiphenyl	37680-65-2				10				Matthews and Zacharewski (2000)
1511	hER	2,4,6-Trichlorobiphenyl	35693-92-6	≥ 99%			100				Vakharia and Gierthy (1999)
1512	hER	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 98					2.4	0.380	Kuiper et al. (1998) [method a]
1513	hER	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 98					4.7	0.672	Kuiper et al. (1998) [method a]
1514	MUC	2',4',6'-Trichloro-4-biphenylol	14962-28-8					0.048	5.37	0.730	Waller et al. (1996)
1515	hER	2',4',6'-Trichloro-4-biphenylol	14962-28-8	≥ 99%	0.079				0.75	-0.127	Vakharia and Gierthy (1999)
1516	MCF-7 cytosol	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 95	2.5	1.2			6.3	0.799	Kramer et al. (1997)
1517	MUC	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 98	0.0420				2.38	0.377	Korach et al. (1988)
1518	MUC	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 98	3.4				0.32	-0.490	Ramamoorthy et al. (1997b)
1519	MCF-7 cytosol	3,3',4'-Trichloro-4-biphenylol	124882-64-0	> 95	3.8	0.1			0.3	-0.523	Kramer et al. (1997)
1520	MUC	3,4',5'-Trichloro-4-biphenylol	4400-06-0	> 98	1.0000				0.10	-1.000	Korach et al. (1988)
1521	RUC	2,4,5-Trichlorophenoxyacetic acid	93-76-5	98			1000				Blair et al. (2000)
1522	RBC	Triethylamine, 2-[<i>p</i> -(6-methoxy-2-phenyl-3-inden-3-yl)phenoxy] hydrochloride	64-96-0						0.00059	-3.229	Korenman (1970)
1523	hER -FP	Triethylene glycol dimethacrylate	109-16-0	95			5000				Hashimoto et al. (2000)
1524	RUC	4,2',4'-Trihydroxychalcone	961-29-5						0.054	-1.270	Fang et al. (2001)
1525	RUC	3,6,4'-Trihydroxyflavone	253195-19-6						0.45	-0.350	Fang et al. (2001)
1526	RUC	6,7,4'-Trihydroxyisoflavone	17817-31-1				100				Fang et al. (2001)
1527	RUC	7,3',4'-Trihydroxyisoflavone	485-63-2						0.0045	-2.350	Fang et al. (2001)
1528	RUC	1,1,2-Triphenylbut-1-ene	63019-13-6						0.01	-2.000	Jordan et al. (1986)
1529	RUC	Triphenylethylene	58-72-0	99	54.5	5.5			0.002	-2.780	Blair et al. (2000)
1530	RUC	Triphenyl phosphate	115-86-6	99			100				Blair et al. (2000)
1531	MCF-7 cells	Tris-4-(chlorophenyl)methane	27575-78-6	94	0.4				0.1	-1.00	Lascombe et al. (2000)
1532	MCF-7 cells	Tris-4-(chlorophenyl)methanol	30100-80-8	94	0.4				0.1	-1.00	Lascombe et al. (2000)

Data Sorted by Substance and Assay

	A	B	D	G	H	I	J	K	L	M	N
1	Assay Type*	Substance	CASRN†	Purity (%)††	IC ₅₀ (μM)**	SD of IC ₅₀ **	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
1533	RUC	Vanillin	121-33-5	99			100				Blair et al. (2000)
1534	RUC	Vinclozolin	50471-44-8	98.2			100				Blair et al. (2000)
1535	RUC	Vinclozolin	50471-44-8					1000	0.0003	-3.523	Waller et al. (1996)
1536	RUC	-Zearalanol	26538-44-3						30	1.480	Fang et al. (2001)
1537	hER	-Zearalanol	42422-68-4					0.0008	16	1.200	Kuiper et al. (1997)
1538	rER	-Zearalanol	42422-68-4					0.0009	14	1.146	Kuiper et al. (1997)
1539	RUC	-Zearalanol	42422-68-4						0.64	-0.190	Fang et al. (2001)
1540	RUC	Zearalanone	5975-78-0						2.1	0.320	Fang et al. (2001)
1541	RUC	-Zearalenol	36455-72-8						43	1.630	Fang et al. (2001)
1542	GST-aERdef	-Zearalenol	36455-72-8		0.0086	0.0021			36	1.556	Matthews et al. (2000)
1543	GST-cERdef	-Zearalenol	36455-72-8		0.0046	0.0009			70	1.845	Matthews et al. (2000)
1544	GST-hER def	-Zearalenol	36455-72-8		0.0061	0.0002			48	1.681	Matthews et al. (2000)
1545	GST-mER def	-Zearalenol	36455-72-8		0.0051	0.0005			53	1.724	Matthews et al. (2000)
1546	GST-rtERdef	-Zearalenol	36455-72-8		0.0013	0.0001			267	2.427	Matthews et al. (2000)
1547	RUC	-Zearalenol	71030-11-0						0.2	-0.700	Fang et al. (2001)
1548	GST-aERdef	-Zearalenol	71030-11-0		0.073	0.018			4.2	0.623	Matthews et al. (2000)
1549	GST-cERdef	-Zearalenol	71030-11-0		0.014	0.001			23	1.362	Matthews et al. (2000)
1550	GST-hER def	-Zearalenol	71030-11-0		0.023	0.003			13	1.114	Matthews et al. (2000)
1551	GST-mER def	-Zearalenol	71030-11-0		0.024	0.016			11	1.041	Matthews et al. (2000)
1552	GST-rtERdef	-Zearalenol	71030-11-0		0.0037	0.0003			91	1.959	Matthews et al. (2000)
1553	GST-aERdef	Zearalenone	17924-92-4		0.027	0.003			12	1.079	Matthews et al. (2000)
1554	GST-cERdef	Zearalenone	17924-92-4		0.0099	0.0011			33	1.519	Matthews et al. (2000)
1555	GST-hER def	Zearalenone	17924-92-4		0.031	0.003			9.3	0.968	Matthews et al. (2000)
1556	GST-mER def	Zearalenone	17924-92-4		0.023	0.005			12	1.079	Matthews et al. (2000)
1557	GST-rtERdef	Zearalenone	17924-92-4		0.0041	0.0008			82	1.914	Matthews et al. (2000)
1558	hER	Zearalenone	17924-92-4						7	0.845	Kuiper et al. (1998) [method a]
1559	hER	Zearalenone	17924-92-4		0.009				10	1.000	Kuiper et al. (1998) [method b]
1560	hER -FP	Zearalenone	17924-92-4		0.059	0.0008			22	1.342	Nikov et al. (2000)
1561	hER	Zearalenone	17924-92-4						5	0.699	Kuiper et al. (1998) [method a]
1562	hER	Zearalenone	17924-92-4		0.0058				18	1.255	Kuiper et al. (1998) [method b]
1563	MCF-7 cytosol	Zearalenone	17924-92-4		0.007				18	1.255	Dodge et al. (1996)
1564	RUC	Zearalenone	17924-92-4					0.0059	44.07	1.644	Waller et al. (1996)
1565	*Numbers in italics were estimated from a graphical representation of the data.										
1566	*GST-aERdef = glutathione-S-transferase fusion proteins consisting of the "def" domain of the lizard (anole); GST-cERdef = glutathione-S-transferase fusion proteins consisting of the "def" domain of the chicken; GST-hER def = glutathione-S-transferase fusion proteins consisting of the "def" domain of the human ER receptor; GST-mER def = glutathione-S-transferase fusion proteins consisting of the "def" domain of the mouse ER receptor; GST-rtERdef = glutathione-S-transferase fusion proteins consisting of the "def" domain of the rainbow trout; hER = semi-purified human ER protein; hER -FP = semi-purified human ER measured using fluorescence polarization; hER = semi-purified human ER protein; MCF-7 cells = intact MCF-7 cells; MCF-7 cytosol = cytosol from human adenocarcinoma cells; MUC = mouse uterine cytosol; RBC = rabbit uterine cytosol; rER = semi-purified rat ER protein; RUC = rat uterine cytosol. HDT = highest dose tested; IC ₅₀ = concentration of the test substance that induces a 50% decrease in binding by the reference estrogen; Ki = dissociation constant of a receptor-ligand complex; RBA = relative binding affinity = (IC ₅₀ test substance/IC ₅₀ reference estrogen) x100; SD = standard deviation.										
1567	**Empty cells indicate that an IC ₅₀ or K _i could not be obtained, in which case the HDT was reported, or that the IC ₅₀ or K _i was not reported but instead the RBA was reported.										
1568	*** Empty cells indicate that an RBA was not provided or could not be calculated because an IC ₅₀ or K _i could not be obtained. Thus no log RBA could be determined.										
1569	† Empty cells indicate that no CASRN could be found.										
1570	†† Empty cells indicate that no information was provided in the publication.										

Appendix D2

Substances Tested in the *In Vitro* ER Binding Assays

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Appendix E

Assay Distribution of Substances Tested in *In Vitro* ER Binding Assays

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Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
4,4'-(1,3-Adamantanediyl)diphenol								1								1
2-(1-Adamantyl)-4-methylphenol	41031-50-9							1								1
4-(1-Adamantyl)phenol	29799-07-3							1								1
Alachlor	15972-60-8						1								1	2
Aldosterone	52-39-1														1	1
Aldrin	309-00-2														1	1
Allethrin	584-79-2							1								1
<i>p</i> -(7-Alloxy)-11-ethyl-dibenzo- <i>[b,f]</i> thiepin-10-yl)phenol	85850-86-8														1	1
<i>p</i> -(3-(Alloxy)-11-ethyl-6H-dibenzo- <i>[b,f]</i> thiocin-12-yl)phenol hemihydrate	85850-88-0														1	1
<i>p</i> -(2-(Alloxy)-6-ethyl-11,12-dihydroxydibenzo- <i>[a,e]</i> cyclooctene-5-yl)phenol	85850-87-9														1	1
3-(Alloxy)-10-ethyl-11-(4-hydroxyphenyl)dibenzo- <i>[b,f]</i> thiepin	85850-85-7														1	1
3-(Alloxy)-11-ethyl-12-phenyl 6H-dibenzo- <i>[b,f]</i> thiocin	85850-84-6														1	1
3-(Alloxy)-10-ethyl-11-phenyldibenzo- <i>[b,f]</i> thiepin	85850-82-4														1	1
3-(Alloxy)-10-ethyl-11-phenyldibenz- <i>[b,f]</i> joxepin	83807-07-2														1	1
3-(Alloxy)-11-ethyl-12-phenyl 5,6-dihydroxydibenzo- <i>[a,e]</i> cyclooctene	85850-83-5														1	1
Amaranth	915-67-3														1	1
2-Aminoestratriene-3,17-diol	107900-30-1										1					1
2-Aminoestratrien-17-ol	17522-06-4										1					1
4-Aminoestratrien-17-ol	17522-04-2										1					1
4-Aminoestratriene-3,17-diol	107900-31-2										1					1
4-Aminophenyl ether	101-80-4														1	1
4- <i>tert</i> -Amylphenol	80-46-6						1		1						1	3
3-Androstenediol	25126-76-5						1							1		2
5-Androstane-3,17-diol	1852-53-5										1	1			1	3
5-Androstane-3,17-diol	571-20-0						1				1	1		1	1	5
5-Androstane-3,17-diol	1851-23-6											1				1
5-Androstenedione	1229-12-5						1							1		2
5-Androstane-3,17-dione	5982-99-0						1					1		1		3
5-Androstane-3-ol-17-one	53-41-8											1				1
4-Androstenediol	1156-92-9						1							1		2
5-Androstenediol	521-17-5						3		2				1	1		4
4-Androstenedione	63-05-8						1				1	1		1		4

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Anthracene	120-12-7									1						1
Apigenin	520-36-5						1		2						1	3
Aroclor 1221	11104-28-2														1	1
Aroclor 1254	11097-69-1														1	1
Atrazine	1912-24-9	1	1	1	1	1		1							2	7
Aurin	603-45-2														1	1
Baicalein	491-67-8														1	1
Benomyl	17804-35-2						1									1
Benzo[a]anthracene	56-55-3			1					1	1						3
Benzeneacetonitrile, a-bis(4-hydroxyphenyl) methylene	66422-14-8									1	1					2
Benzo[a]carbazole	239-01-0			1					1							2
Benzo[c]carbazole				1					1							2
Benzo[b]fluoranthene	205-99-2									1						1
Benzo[k]fluoranthene	207-08-9									1						1
Benzo[a]fluorene	238-84-6														1	1
Benzo[b]fluorene	243-17-4			1					1							2
Benzo[b]naphtho[2,1-d]thiophene	239-35-0			1					1							2
Benzo[b]naphtho[2,3-d]thiophene	243-46-9			1					1							2
Benzo[ghi]perylene	191-24-2									1						1
Benzo[c]phenanthrene	195-19-7			1					1							2
Benzo[e]pyrene	192-97-2									1						1
Benzo[a]pyrene	50-32-8									1						1
Benzyl alcohol	100-51-6														1	1
4-Benzylloxyphenol	103-16-2														1	1
Benzylparaben	94-18-8														1	1
Biochanin A	491-80-5						1		1						1	3
Bis(m -acetoxy)-1,1,2-triphenylbut-1-ene	100808-56-8														1	1
Bis(p -acetoxy)-1,1,2-triphenylbut-1-ene	100808-54-6														1	1
Bisdesoxyestradiol	1217-09-0														1	1
1,1-Bis-(4-hydroxyphenyl) ethane	2081/8/5														1	1
4,4-Bis(4-hydroxyphenyl) heptane	7425-79-8														1	1
3,4-Bis(3-hydroxyphenyl)hexane	68266-24-0									1	1					2
3,3-Bis(4-hydroxyphenyl) pentane	3600-64-4														1	1
1,1-Bis(4-hydroxyphenyl) propane	1576-13-2														1	1
2,2-Bis(4-hydroxyphenyl) propanol	142648-65-5														1	1
(2,2-Bis(p -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	1	1	1	1	1	1	1	1			1			3	10
Bisphenol A	80-05-7	1	1	1	1	1	3	2	2	1	1	1	1	1	5	14

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Bisphenol A bis(chloroformate)	2024-88-6														1	1
Bisphenol A diglycidyl ether	1675-54-3														2	1
Bisphenol A diglycidyl ether dimethacrylate	1565-94-2							1							2	2
Bisphenol A dimethacrylate	3253-39-2												1		2	2
Bisphenol A ethoxylate	68140-85-2														1	1
Bisphenol A ethoxylate diacrylate	64401-02-1														1	1
Bisphenol A glucuronide												1				1
Bisphenol A propoxylate	37353-75-6														1	1
Bisphenol AF	1478-61-1														1	1
Bisphenol B	77-40-7														2	1
Bisphenol C	79-97-0														1	1
Bisphenol C 2	14868-03-2									1	1				1	3
Bisphenol E	6052-84-2														1	1
2,2'-Bisphenol F	2467-02-9														1	1
4,4'-Bisphenol F	620-92-8														2	1
Bisphenol S	80-09-1														1	1
16 -Bromo-17 -estradiol	54982-79-5						2		2							2
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl) phenoxy]-	107144-85-4									1	1					2
1,3-Butanediol, 4-[4-[1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthalenyl)phenoxy]-	107163-56-4									1	1					2
Butolame	150748-23-5														1	1
Butyl 4-aminobenzoate	94-25-7														1	1
<i>n</i> -Butylbenzene	104-51-8														1	1
<i>sec</i> -Butylbenzene	135-98-8														1	1
Butyl benzyl phthalate	85-68-7	1	1	1	1	1		2					1		5	8
Butylparaben	94-26-8														2	1
2- <i>sec</i> -Butylphenol	89-72-5														1	1
2- <i>tert</i> -Butylphenol	88-18-6														1	1
3- <i>tert</i> -Butylphenol	585-34-2														1	1
4- <i>sec</i> -Butylphenol	99-71-8														1	1
4- <i>tert</i> -Butylphenol	98-54-4						1		1						2	3
Butyl phthalyl <i>n</i> -butyl glycolate	85-70-1							1								1
Caffeine	58-08-2														1	1
Carbaryl	63-25-2														1	1
Carbofuran	1563-66-2														1	1
Castor oil	8001-79-4														1	1
(\pm)-Catechin	7295-85-4														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Chalcone	94-41-7														1	1
Chlordane	57-74-9						1									1
-Chlordane	5103-71-9														1	1
Chlormequat chloride	999-81-5												1			1
2-Chloro-4-amino-6-isopropylamino-1,3,5-triazine	6190-65-4							1								1
2'-Chloro-4,4'-biphenyldiol	56858-70-9											1				1
2-Chloro-4-biphenylol	23719-22-4											1			1	2
4-Chloro-4'-biphenylol	28034-99-3											2			1	2
2-Chloro-4,6-diamino- <i>S</i> -triazine	3397-62-4														1	1
2-Chloro-4-ethylamino-6-amino-1,3,5-triazine	1007-28-9							1								1
2-Chloro-4-ethylamino-6-(1-hydroxyisopropyl)amino-1,3,5-triazine	142179-80-4							1								1
2-Chloro-4-isopropylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine	142200-36-0							1								1
11 -Chloromethylestradiol	71794-60-0									1	1					2
2-Chloro-4-methylphenol	6640-27-3														1	1
4-Chloro-2-methylphenol	1570-64-5														1	1
4-Chloro- <i>m</i> -cresol	59-50-7														1	1
2-Chlorophenol	95-57-8														1	1
4-Chlorophenol	106-48-9														1	1
Chlorotamoxifen	77588-46-6														1	1
Cholesterol	57-88-5														1	1
Chrysene	218-01-9			1					1	1					1	4
Chrysin	480-40-0						1		1						1	3
Cineole	470-82-6														1	1
Cinnamic acid	621-82-9														1	1
<i>cis</i> -Clomiphene	15690-55-8												1			1
<i>trans</i> -Clomiphene	911-45-5						1						1	1		3
Clomiphene citrate	50-41-9														1	1
Colchicine	64-86-8												1			1
Corticosterone	50-22-6						1							1	1	3
Cortisol	50-23-7											1				1
Coumestrol	479-13-0	1	1	1	1	1	3	1	2		1			1	2	11
<i>p</i> -Cumyl phenol	599-64-4														1	1
Cyclofenil diphenol	5189-40-2									1	1					2
Cycloprop[14 <i>R</i> ,15]estra-1,3,5(10)-triene-3,17-diol, 3',15-dihydro-	73860-54-5									1	1					2

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Cycloprop[14S ,15]estra-1,3,5(10)-triene-3,17 -diol, 3', 15-dihydro-	105455-76-3									1	1					2
Cypermethrin	52315-07-8							1								1
Daidzein	486-66-8						3	1	3						1	4
<i>m,p'</i> -DDD	4329-12-8														1	1
<i>o,p'</i> -DDD	53-19-0						2		1						2	3
<i>p,p'</i> -DDD	72-54-8						2		1						3	3
<i>o,p'</i> -DDE	3424-82-6	1	1	1	1	1	1		1						2	8
<i>p,p'</i> -DDE	72-55-9	1	1	1	1	1	1		1				1		3	9
<i>o,p'</i> -DDT	789-02-6	1	1	1	1	1	2	1	1		1	1	1		5	12
(-)- <i>o,p'</i> -DDT	58633-26-4									1					1	2
(+)- <i>o,p'</i> -DDT	58633-27-5									1					1	2
<i>p,p'</i> -DDT	50-29-3	1	1	1	1	1	1	1	1						3	9
Dehydroepiandrosterone	53-43-0	1	1	1	1	1	1							1		7
14-Dehydroestradiol-17	58699-19-7														1	1
9, 11-Dehydroestradiol											1					1
14-Dehydroestradiol-17 3-methyl ether	35664-58-7														1	1
14-Dehydroestrone	2119-18-8														1	1
14-Dehydroestrone 3-methyl ether	17550-11-7														1	1
3-Deoxyestradiol	2529-64-8									1	1				1	3
3-Deoxyestrone	53-45-2														1	1
(<i>R</i>)-4'-Deoxyindenestrol A	138515-00-1											1				1
(<i>R</i>)-5-Deoxyindenestrol A	138515-02-3											1				1
(<i>rac</i>)-4'-Deoxyindenestrol A												1				1
(<i>rac</i>)-5-Deoxyindenestrol A	138472-84-1											1				1
(<i>S</i>)-4'-Deoxyindenestrol A	138514-99-5											1				1
(<i>S</i>)-5-Deoxyindenestrol A	138515-01-2											1				1
17-Desoxyestradiol	53-63-4									1	1				3	3
Dexamethasone	50-02-2														1	1
1,3-Diacetoxy-17 -ethinyl-7 -methyl-1,3,5(10)-estratrien-17 -ol															1	1
4,4'-Diaminostilbene dihydrochloride	66635-40-3														1	1
Dibenz[<i>a,h</i>]anthracene	53-70-3									1						1
Dibenzo-18-crown-6	14187-32-7														1	1
1,3-Dibenzoyloxy-17 -ethinyl-7 -methyl-1,3,5(10)-estratrien-17 -ol															1	1
1,3-Dibenzyltetramethyldisiloxane															1	1
Dibutyl benzyl phthalate		1	1	1	1	1										5
2,6-Di- <i>tert</i> -butylphenol	128-39-2														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Dibutyl phthalate	84-74-2							1					1		3	3
2,4'-Dichlorobiphenyl	34883-43-7														1	1
2,5-Dichlorobiphenyl	34883-39-1						1									1
3,4-Dichlorobiphenyl	2974-92-7						1									1
3,5-Dichlorobiphenyl	34883-41-5						1									1
4,4'-Dichlorobiphenyl	2050-68-2														1	1
2,5-Dichloro-2'-biphenylol	53905-30-9						1									1
2,5-Dichloro-3'-biphenylol	53905-29-6						1									1
2',5'-Dichloro-4-biphenylol	53905-28-5						1					2			1	3
2',6'-Dichloro-4-biphenylol	79881-33-7											1				1
3,4-Dichloro-2'-biphenylol	209613-97-8						1									1
3,4-Dichloro-3'-biphenylol	14962-34-6						1									1
3,4-Dichloro-4'-biphenylol	53890-77-0						1									1
3,5-Dichloro-2'-biphenylol							1									1
3,5-Dichloro-4'-biphenylol							1									1
3,5-Dichloro 2-hydroxy-2-methylbut-3-enalide	16776-82-1														2	1
2,4-Dichlorophenoxyacetic acid	94-75-7														1	1
2-[[[(3,5-Dichlorophenyl)amino]-carbonyl]oxy]-2-methyl-3-butenic acid	119209-27-7														2	1
Dieldrin	60-57-1						1	1				1			2	4
Dienestrol	84-17-3						1							1	1	3
-Dienestrol	13029-44-2											1				1
-Dienestrol	35495-11-5											1				1
1,3-Diethyl-6,4'-dihydroxy-2-phenylindene															1	1
Di 2-ethylhexyl adipate	103-23-1							1								1
Diethylhexyl phthalate	117-81-7							1							2	2
1,3-Diethyl-4-hydroxy-2-phenylindene															1	1
1,3-Diethyl-6-hydroxy 2-phenylindene															1	1
meso-p -(, -Diethyl-p -methylphenethyl)phenol	267408-76-4														1	1
Diethyl phthalate	84-66-2														1	1
Diethylstilbestrol	56-53-1	1	1	1	1	1	3	4	2	1	2	7	2	1	11	14
3,3'-Diethylstilbestrol	5959-71-7									1	1					2
Diethylstilbestrol dimethyl ether	130-79-0														1	1
Diethylstilbestrol epoxide	6052-82-0											1				1
Diethylstilbestrol-phenanthrene												1				1
5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (rac)							1		1							2
5,11-trans -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
(5R,11R)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
(5S,11S)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
Diethyl phthalate	84-75-3														1	1
5,6-Dihydro-8-[2-(dimethylamino)ethoxy]-12-ethyl-11-phenyl-dibenzo[a,e]cyclooctene, hydrate (1:4)	85850-78-8														1	1
Dihydrogenistein	21554-71-2						1		1							2
Dihydroglycitein	94105-88-1								1							1
5 α -Dihydrotestosterone	521-18-6	1	1	1	1	1	1	1				1		1	2	10
5 β -Dihydrotestosterone	571-22-2														1	1
2,2'-Dihydroxybenzophenone	835-11-0														1	1
2,4-Dihydroxybenzophenone	131-56-6														1	1
4,4'-Dihydroxybenzophenone	611-99-4														1	1
4,4'-Dihydroxybiphenyl	92-88-6						1		1			1				3
Dihydroxydiethylstilbestrol	7507-01-9											1				1
6,4'-Dihydroxyflavone	63046-09-3														1	1
3,3'-Dihydroxyhexestrol	79199-51-2														1	1
2,2'-Dihydroxy-4-methoxybenzophenone	131-53-3														1	1
3-(2,3-Dihydroxypropoxy)-10-ethyl-11-phenyldibenz[b,f]oxepin	85850-89-1														1	1
Diisobutyl phthalate	84-69-5														1	1
Diisodecyl phthalate	26761-40-0														1	1
Diisoheptyl phthalate	41451-28-9														1	1
Diisononyl phthalate	28553-12-0														2	1
11-[2-(N,N-Dimethylamino)ethoxy]estra-1,3,5 (10)-triene-3,17 β -diol															1	1
3-[2-(Dimethylamino)ethoxy]-11-ethyl-12-phenyl-6H-dibenzo[b,f]thiactin	85850-79-9														1	1
3-[2-(Dimethylamino)ethoxy]-10-ethyl-11-phenyldibenz[b,f]oxepin	85850-76-6														1	1
7-[2-(Dimethylamino)ethoxy]-11-ethyl-10-phenyldibenz[b,f]thiepin	85850-77-7														1	1
11-[3-(N,N'-Dimethylamino)propoxy]estra-1,3,5 (10)-triene-3,17 β -diol	130043-38-8														1	1
1 α -Dimethyl- β -ethylallenolic acid	15372-37-9														1	1
2,6-Dimethylhexestrol	334707-28-7														1	1
1,6-Dimethylnaphthalene	575-43-9														1	1
Dimethyl phthalate	131-11-3														1	1
1 α -Dimethylstilbestrol	552-80-7												1		1	2
Dimethyl sulfoxide	2206-27-1									1						1
5,11-trans-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
(5 <i>R</i> ,11 <i>R</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
(5 <i>S</i> ,11 <i>S</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
Di- <i>n</i> -octyl phthalate	117-84-0														2	1
Diphenolic acid	126-00-1														1	1
<i>trans</i> , <i>trans</i> -1,4-Diphenyl-1,3-butadiene	886-65-7														1	1
4-[1,2-(Diphenyl-1-butenyl)]phenol acetate	100808-55-7														1	1
2,3-Diphenylindenone-1															1	1
4-[1-(Diphenylmethylene)propyl]phenol acetate	82333-68-4														1	1
1,3-Diphenyltetramethyldisiloxane	56-33-7														1	1
5,11- <i>trans</i> -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
(5 <i>R</i> ,11 <i>R</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
(5 <i>S</i> ,11 <i>S</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
4-Dodecylphenol	104-43-8														1	1
Doisynoesol	15372-34-6														1	1
Dopamine	51-61-6														1	1
Droloxifene	82413-20-5									1	1				1	3
Empenthrin	54406-48-3							1								1
- Endosulfan	959-98-8	1	1	1	1	1	1									6
-Endosulfan	115-29-7						1		1			1	1		2	5
-Endosulfan	33213-65-9	1	1	1	1	1	1				1					7
16-Epiestriol	547-81-9												1			1
17-Epiestriol	1228-72-4						1		1							2
Epitestosterone	481-30-1														1	1
Equilenin	517-09-9												1			1
Equilin	474-86-2												1			1
Equol	531-95-3						1		1						1	3
Erythro-MEA	20576-52-7												1			1
16-Estradiol	1090-04-6										1		1			2
17-Estradiol	57-91-0						2		1		1	1	1	1	2	7
17-Estradiol	50-28-2	2	1	3	1	2	13	7	10	4	6	12	2	1	19	14
9-Estradiol											1					1
Estradiol 17-acetate													1			1
17-Estradiol 3-acetate	4245-41-4												1			1
Estradiol 3-benzoate	50-50-0	1	1	1	1	1										5
Estradiol diacetate	3434-88-6												1			1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
17 -Estradiol 3-methyl ether	1035-77-4												1		1	2
9-Estratetraene-3,17 -diol	791-69-5									1	1					2
Estra-1,3,5(10),6-tetraen-17-one, 3-hydroxy-													1			1
Estra-1,3,5(10)-triene-3,17 -diol, 14,15 -epoxy-	79581-12-7									1	1					2
Estra-1,3,5(10)-triene-3,17 -diol, 14 ,15 -epoxy-	79645-49-1									1	1					2
Estra-1,3,5,(10),trien-3,14,17 -triol	16288-09-8									1	1					2
Estratriene-3,6 ,17 -triol	1229-24-9														1	1
Estriol	50-27-1	1	1	1	1	1	1			1	3	1	1	1	2	12
Estrone	53-16-7	1	1	1	1	1	1	1		1	3	1	1	1	4	13
Estrone 3-acetate	901-93-9												1			1
Estrone 3-methyl ether	1624-62-0														1	1
Estrone 3-sulfate	481-97-0						1							1		2
17 -Ethinyl estradiol	57-63-6	1	1	1	1	1							1		4	7
17 -Ethinyl estradiol	4717-38-8														1	1
Ethyl cinnamate	103-36-6														1	1
3-Ethyl-6,4'-dihydroxy-2-phenylindene															1	1
2-Ethylhexyl paraben	5153-25-3														1	1
3-Ethyl-4'-hydroxy 2-phenylindene-1															1	1
3-Ethyl-6-hydroxy 2-phenylindene-1															1	1
4-Ethyl-7-hydroxy-3-(4-methoxyphenyl)-2H -1-benzopyran-2-one	5219-17-0														1	1
3-[(10-Ethyl-11- <i>p</i> -hydroxyphenyl)dibenzo- <i>[b,f]</i>]oxepin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)	85850-93-7														1	1
3-[(10-Ethyl-11- <i>p</i> -hydroxyphenyl)dibenzo- <i>[b,f]</i>]thiepin-3-yl)oxy]-1,2-propanediol	85850-94-8														1	1
3-[(11-Ethyl-12-(<i>p</i> -hydroxyphenyl)-6- <i>H</i> -dibenzo- <i>[b,f]</i>]thiocin-3-yl)oxy]-, hemihydrate 1,2-propanediol	85864-54-6														1	1
3-[(6-Ethyl-5-(<i>p</i> -hydroxyphenyl)-11,12-dihydrodibenzo- <i>[a,e]</i>]cycloocten-2-yl)oxy]-1,2-propanediol	85850-95-9														1	1
3-Ethyl-4'-hydroxy-2-phenylindene															1	1
3-Ethyl-6-hydroxy 2-phenylindene															1	1
3-Ethyl-4-(<i>p</i> -methoxyphenyl)-2-methyl-3-cyclohexene-1-carboxylic acid	1755-52-8												1			1
Ethyl paraben	120-47-8														1	1
2-Ethylphenol	90-00-6														1	1
3-Ethylphenol	620-17-7														1	1
4-Ethylphenol	123-07-9														1	1
3-[(10-Ethyl-11-phenyl)dibenzo- <i>[b,f]</i>]thiepin-3-yl)oxy]-1,2-propanediol, complexed with isopropyl alcohol 2:1	85850-90-4														1	1
3-[(11-Ethyl-12-phenyl-6- <i>H</i> -dibenzo- <i>[b,f]</i>]thiocin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)	85850-92-6														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
3-[6-Ethyl-5-phenyl-11,12-dihydrodibenzo[<i>a,e</i>]cycloocten-2-yl]oxy]-1,2-propanediol	85850-91-5														1	1
Eugenol	97-53-0														1	1
Fenvalerate	51630-58-1							1								1
Fisetin	528-48-3														1	1
Flavanone	17002-31-2														1	1
Flavone	525-82-6						1		1						1	3
Fluoranthene	206-44-0									1						1
Fluorene	86-73-7									1						1
4-Fluoroestratrien-17 -ol	96607-54-4										1					1
2-Fluoroestratrien-17 -ol	101772-22-9										1					1
2-(2-Fluorophenyl)-3-phenyl-6-hydroxyindene															1	1
Fluorotamoxifen	73617-96-6														1	1
Folic acid	59-30-3														1	1
Formononetin	485-72-3						1		1						1	3
Furfural	98-01-1														1	1
Genistein	446-72-0	1	1	1	1	1	4	1	3		2			1	2	11
Genistin	529-59-9								1						1	2
Glyceollin	66241-09-6							1								1
Glycitein	40957-83-3						1		1							2
Glycitin									1							1
Heptachlor	76-44-8														1	1
2,2',3,3',4',5',6'-Heptachlorobiphenyl	52663-70-4	1		1		1										3
2,2',3,3',4',5,6'-Heptachlorobiphenyl	68194-16-1	1		1		1										3
2,2',3,3',5,5',6'-Heptachlorobiphenyl	52663-64-6	1		1		1										3
2,2',3,4,4',5',6'-Heptachlorobiphenyl	52663-69-1	1		1		1										3
2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3	1		1		1										3
2,2',3,4',5,5',6'-Heptachlorobiphenyl	52663-68-0	1		1		1										3
2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7	1		1		1										3
2,3,3',4,4',5,6'-Heptachlorobiphenyl	41411-64-7	1		1		1										3
2,3,3',4',5,5',6'-Heptachlorobiphenyl	69782-91-8	1		1		1										3
2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	1		1		1	1		1							5
2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	1		1		1	1		1							5
2,2',3,4',5,5',6'-Heptachloro-4-biphenylol	158076-68-7						1		1							2
Heptanal	111-71-7														1	1
4-(Heptyloxy)phenol	13037-86-0														1	1
Heptyl 4-paraben	1085-12-7														1	1
Hesperetin	520-33-2														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Hexachlorobenzene	118-74-1														1	1
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	1		1		1										3
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	1		1		1										3
2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0	1		1		1										3
2,2',3,5,5',6'-Hexachlorobiphenyl	52663-63-5	1		1		1										3
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	1		1		1										3
2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2						1					1				2
2,2',3,4',5,6'-Hexachlorobiphenyl	38380-04-0	1		1		1										3
2,3,3',4,4',6'-Hexachlorobiphenyl	74472-42-7	1		1		1										3
2,3',4,4',5',6'-Hexachlorobiphenyl	59291-65-5	1		1		1										3
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	1		1		1										3
2,2',3,3',4',5'-Hexachloro-4-biphenylol	158076-62-1	1		1		1	1		1							5
2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	1		1		1	1		1							5
2',3,3',4',5,5'-Hexachloro-4-biphenylol	158076-63-2										1					1
<i>n</i> -Hexanol	111-27-3														1	1
Hexestrol	84-16-2						1			1	1		1	1	2	6
<i>DL</i> -Hexestrol	5776-72-7														1	1
Hexestrol monomethyl ether	13026-26-1														1	1
3-Hydroxybenzo[<i>b</i>]naphtho[2,1- <i>d</i>]thiophene				1					1							2
2-Hydroxybenzo[<i>c</i>]phenanthrene	22717-94-8			1					1							2
3-Hydroxybenzo[<i>b</i>]phenanthro[2,3- <i>d</i>]thiophene				1					1							2
4'-Hydroxychalcone	2657-25-2														1	1
4-Hydroxychalcone	20426-12-4														1	1
4'-Hydroxychalcone (<i>cis</i> - and <i>trans</i> -)	38239-52-0														1	1
2-Hydroxychrysene	65945-06-4			1					1							2
4'-Hydroxy-2,3-diphenylindenone-1															1	1
6'-Hydroxy-2,3-diphenylindenone-1															1	1
11 -Hydroxyestradiol	5444-22-4									1	2		1		1	4
11 -Hydroxyestradiol	1464-61-5										1					1
14 -Hydroxyestradiol	60183-66-6									1	1					2
2-Hydroxyestradiol	362-05-0						1							1		2
4-Hydroxyestradiol	5976-61-4						1							1		2
2-Hydroxyestratrien-17 -ol	2259-89-4										1					1
4-Hydroxyestratrien-17 -ol	17592-89-1										1					1
3-Hydroxyestra-1,3,5(10)-trien-16-one	3601-97-6														1	1
2-Hydroxyestrone	362-06-1						1		1							2
2-Hydroxyethyl methacrylate	868-77-9							1								1
3'-Hydroxyflavanone	92496-65-6														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
4'-Hydroxyflavanone	135413-27-3														1	1
6-Hydroxyflavanone	4250-77-5														1	1
7-Hydroxyflavanone	6515-36-2														1	1
6-Hydroxyflavone	6665-83-4														1	1
7-Hydroxyflavone	6665-86-7														1	1
Hydroxyflutamide	52806-53-8														2	1
6-Hydroxy-2'-methoxyflavone	61546-59-6														1	1
2-Hydroxy-4-methoxybenzophenone	131-57-7														1	1
2-Hydroxy-5-methylchrysene				1					1							2
8-Hydroxy-5-methylchrysene				1					1							2
16 -Hydroxy-16-methyl-17 -estradiol 3-methyl ether	3434-79-5														1	1
4-Hydroxytamoxifen	68047-06-3	1	1	1	1	1	3	3	2	1	1	1		1	1	13
6-Hydroxytetralin	1125-78-6														1	1
ICI 164384	98007-99-9	1	1	1	1	1	2		1					1	1	9
ICI 182780	129453-61-8						1		1				1		2	4
Imiprophrin	72963-72-5							1								1
Indanestrol	71855-45-3											2				1
(S)-Indenestrol B												1				1
Indanyldiethylstilbestrol												1				1
Indenestrol A	24643-97-8											3				1
(R)-Indenestrol A	115217-03-3											1				1
(S)-Indenestrol A	115217-04-4											1				1
Indenestrol B	38028-27-2											2				1
(R)-Indenestrol B	115217-06-6											1				1
(rac)-Indenestrol B	133830-97-4											1				1
Indeno[1,2,3- <i>cd</i>]pyrene	193-39-5									1						1
Indole[3,2- <i>b</i>]carbazole															1	1
16 -Iodoestradiol	71765-94-1										1					1
(E)-17 -Iodovinylestradiol	82123-96-4										1				1	2
(Z)-17 -Iodovinylestradiol	177159-09-0										1				1	2
Ipriiflavone	35212-22-7						1		1							2
Isoeugenol	97-54-1														1	1
Kaempferol	520-18-3						1		2						1	3
Kepone	143-50-0	1	1	1	1	1	1	1	1			1			3	10
11-Keto-9 -estradiol											1					1
16-Ketoestradiol	566-75-6						1		1		1		1			4
6-Ketoestradiol	571-92-6									1	1					2
Lindane	58-89-9														2	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Melatonin	73-31-4														1	1
MER-25	67-98-1											1			1	
Mestilbol	18839-90-2														1	1
Mestranol	72-33-3												1		2	2
Methoxychlor (<i>p,p'</i> -)	72-43-5	1	1	1	1	1	2	1	1		1	1	1	1	5	13
Methoxychlor olefin	2132-70-9														1	1
3-Methoxyestriol	1474-53-9													1	1	
<i>E</i> -11 -Methoxy-17 -iodovinylestradiol	90857-55-9										1				1	2
<i>Z</i> -11 -Methoxy-17 -iodovinylestradiol	177159-11-4										1				1	2
3-Methoxy-10-methyl-11-phenyldibenzo[<i>b,f</i>]thiepin															1	1
Methoxytamoxifen															1	1
7 -Methyl-14-Dehydroestradiol-17	88598-62-3														1	1
9 -Methyl-14-Dehydroestradiol-17	88598-63-4														1	1
11 -Methyl-14-dehydroestradiol-17 3-methyl ether	88598-65-6														1	1
7 -Methyl-14-dehydroestradiol-17 3-methyl ether	35644-59-8														1	1
9 -Methyl-14-dehydroestradiol-17 3-methyl ether	88598-64-5														1	1
7 -Methyl-14-dehydroestrone	88958-66-7														1	1
9 -Methyl-14-dehydroestrone	88598-67-8														1	1
11 -Methyl-14-dehydroestrone 3-methyl ether	88598-69-0														1	1
7 -Methyl-14-dehydroestrone 3-methyl ether	35644-57-6														1	1
9 -Methyl-14-dehydroestrone 3-methyl ether															1	1
4,4'-Methylenebis(<i>N,N</i> -dimethylbenzeneamine)	101-61-1														1	1
4,4'-Methylenedianiline	101-77-9														1	1
11 -Methylestradiol-17	23637-93-6									1	1				1	3
7 -Methylestradiol-17	10448-97-2														1	1
9 -Methylestradiol-17	66463-44-3														1	1
11 -Methylestradiol-17 3-methyl ether	18046-75-8														1	1
7 -Methylestradiol-17 3-methyl ether	15506-01-1														1	1
9 -Methylestradiol-17 3-methyl ether	51242-32-1														1	1
11 -Methylestrone	13667-06-6														1	1
7 -Methylestrone	10448-96-1														1	1
9 -Methylestrone	71563-77-4														1	1
11 -Methylestrone 3-methyl ether	13667-04-4														1	1
7 -Methylestrone 3-methyl ether	10449-00-0														1	1
9 -Methylestrone 3-methyl ether	31266-41-8														1	1
1-Methyl-3-ethyl-6,4'-dihydroxy-2-phenylindene															1	1
1-Methyl-6-hydroxy-2,3-diphenylindene															1	1
Methyl methacrylate	80-62-6							1								1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Methyl paraben	99-76-3														1	1
2-(2-Methylphenyl)-3-phenyl-6-hydroxyindene															1	1
Methyltamoxifen	73617-95-5														1	1
Methoxychlor (<i>p,p'</i> + <i>o,p'</i> -)	30667-99-3														1	1
Metolachlor	51218-45-2														1	1
Mirex	2385-85-5														1	1
Monohydroxytamoxifen	68392-35-8														1	1
Mono- <i>m</i> -acetoxy-1,1,2-triphenylbut-1-ene	82333-69-5														1	1
Monohydroxymethoxychlor	28463-03-8														1	1
Monohydroxymethoxychlor olefin	75938-34-0														1	1
Morin	480-16-0														1	1
Moxestrol	34816-55-2						1					1		1	1	4
Myricetin	529-44-2														1	1
Nafoxidine	1845-11-0						1			1	1			1	1	5
Naringenin	480-41-1	1	1	1	1	1	1		2						1	8
Naringin	10236-47-2														1	1
Nerolidol	7212-44-4														1	1
2-Nitroestratriene-3,17 -diol	6298-51-7										1					1
4-Nitroestratriene-3,17 -diol	6936-94-3										1					1
2-Nitroestratrien-3-ol-17-one	5976-73-8										1					1
4-Nitroestratrien-3-ol-17-one	5976-74-9										1					1
Nitromifene	10448-84-7												1			1
<i>cis</i> -Nonachlor	5103-73-1						1									1
<i>trans</i> -Nonachlor	39765-80-5						1									1
Nonylbenzene	1081-77-2														1	1
<i>p</i> -Nonylphenol	104-40-5							2		1	1				10	4
<i>n</i> -Nonylphenol	25154-52-3						2		2			1	1			4
Nonylphenol dodecylethoxylate													1			1
Nordiethylguaiaretic acid	500-38-9														1	1
Norethindrone	68-22-4						1							1		2
Norethynodrel	68-23-5						1							1	2	3
19-Nortestosterone	434-22-0						1					1		1		3
2,2',3',4',5',5'-Octachlorobiphenyl	35694-08-7	1		1		1										3
1,8-Octanediol	629-41-4														1	1
4- <i>n</i> -Octylphenol	1806-26-4						1		1	1	1		1		1	6
4- <i>tert</i> -Octylphenol	140-66-9	1	1	1	1	1	1	1	1						3	9
2,2',3,3',6-Pentachlorobiphenyl	52663-60-2	1		1		1										3
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	1		1		1										3

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
2,2',3,4',6-Pentachlorobiphenyl	68194-05-8	1		1		1										3
2,2',3,5',6-Pentachlorobiphenyl	38379-99-6	1		1		1										3
2,2',4,4',5-Pentachlorobiphenyl	38380-01-7	1		1		1										3
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	1		1		1										3
2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8	1		1		1						1				4
2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9	1		1		1										3
2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1	1		1		1										3

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
3,3',4,4',5',-Pentachlorobiphenyl	57465-28-8	1		1		1										3
2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4						1		1			1			1	4
2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2						1		1			1			1	4
2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3						1		1			1			1	4
2,2',4,6,6'-Pentachloro-4-biphenylol												1				1
2',3,3',4,4'-Pentachloro-2-biphenylol	150975-80-7										1					1
2,3,3',4',5'-Pentachloro-4-biphenylol	152969-11-4	1		1		1	1		1		1					6
2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9						1		1			1			1	4
2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3						1		1			1			1	4
2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6						1		1			1			1	4
2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7						1		1			1			1	4
2,3',4,4',5'-Pentachloro-3-biphenylol	170946-11-9										1					1
2',3',4,4',5'-Pentachloro-3-biphenylol	150975-81-8										1					1
2',3,4',5,5'-Pentachloro-4-biphenylol	149589-56-0										1					1
2,2',4,6,6'-Pentachloro-4-biphenylol												1				1
3,3',4',5,5'-Pentachloro-4-biphenylol	130689-92-8										1					1
Pentolame	150748-24-6														1	1
Permethrin	52645-53-1							1								1
Phenanthrene	85-01-8									1						1
4-Phenethylphenol	6335-83-7														1	1
Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis	107144-81-0									1	1					2
Phenol, 3-[2-dimethylamino-ethoxy]-10-ethyl-4-hydroxy-phenyl dibenzo[<i>b,f</i>]oxepin	85850-80-2									1	1				1	3
Phenol, 4-[7-(2 dimethylamino)ethoxy]-11-ethylidibenzo[<i>b,f</i>]thiepin-10-yl)	85850-74-4									1	1				1	3
Phenol, 4-[3-(2 dimethylamino)ethoxy]-11-ethylidibenzo[<i>b,f</i>]thioctin-12-yl)-, hydrate (4:1)	85850-81-3									1	1				1	3
Phenol, 4-[2-(2 dimethylamino)ethoxy]-6-ethyl-11,12-dihydrodibenzo[<i>a,e</i>]cycloocten-5-yl]	85850-75-5									1	1				1	3
Phenol, 4-[1-[4-[2-(dimethylamino)ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (<i>E</i>)-	96474-35-0									1	1					2
Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9									1	1					2
Phenol, 4,(1Z) 1-2-diphenyl-1-butenyl)-	69967-80-2									1	1					2
Phenol, 4,4'-[2-phenyl-1-butenylidene]bis	91221-46-4									1	1					2
Phenolphthalein	77-09-8														1	1
Phenolphthalin	81-90-3														1	1
Phenol Red	143-74-8														1	1
-Phenothrin	26002-80-2							1								1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
2-Phenyl-3-(2-fluoro-4-hydroxyphenyl)-6-hydroxyindene															1	1
2-Phenyl-3-(2-fluorophenyl)-6-hydroxyindene															1	1
3-Phenyl-4'-hydroxy-2-phenylindene															2	1
3-Phenyl-6-hydroxy-2-phenylindene															1	1
2-Phenyl-3-(2-methylphenyl)-6-hydroxyindene															1	1
2-Phenyl-3-(4-methylphenyl)-6-hydroxyindene															1	1
2-Phenylphenol	90-43-7														1	1
3-Phenylphenol	580-51-8														1	1
4-Phenylphenol	92-69-3											1			1	2
Phloretin	60-82-2						1		1						1	3
Prallethrin	23031-36-9							1								1
Progesterone	57-83-0						2		1			1		1	3	5
Prolame	99876-41-2														1	1
Promegestone	34184-77-5											1			2	2
Prometon	1610-18-0														1	1
Propazine	139-40-2							1								1
Propyl paraben	94-13-3														1	1
Propylpyrazoletriol							1		1							2
Prunetin	552-59-0														1	1
Pseudodiethylstilbestrol	39011-86-4											2				1
Pyrene	129-00-0									1						1
Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (E)	77413-87-7									1	1					2
Quercetin	117-39-5	1	1	1	1	1	1		1		1				1	9
6-Quinololinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1									1	1					2
7-Quinololinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2									1	1					2
Raloxifene	84449-90-1						2		2							2
Raloxifene hydrochloride	82640-04-8						1									1
Resveratrol	501-36-0														1	1
Rutin	153-18-4														1	1
Simazine	122-34-9	1	1	1	1	1		1							1	7
-Sitosterol	83-46-5	1	1	1	1	1	1							1	1	8
4,4'-Stilbenediol	659-22-3														1	1
4-Stilbenol	3839-46-1														1	1
Suberic acid	505-48-6														1	1
Tamoxifen	10540-29-1	1	1	1	1	1	3	2	2	1	1	1	1	1	4	14
Tamoxifen citrate	54965-24-1														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Taxifolin	480-18-2														1	1
Testosterone	58-22-0						2	1	1			1	1	1	2	7
2,2',3,3'-Tetrachlorobiphenyl	3844-93-8	1		1		1										3
2,2',3,4'-Tetrachlorobiphenyl	52663-59-9	1		1		1										3
2,2',3,6'-Tetrachlorobiphenyl	41464-47-5	1		1		1										3
2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	1		1		1									1	4
2,2',4,5'-Tetrachlorobiphenyl	41464-40-8	1		1		1										3
2,2',4,6'-Tetrachlorobiphenyl	68194-04-7	1		1		1										3
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3						1									1
2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	1		1		1	2		1							5
2,3,3',5'-Tetrachlorobiphenyl	41464-49-7	1		1		1										3
2,3,4,4'-Tetrachlorobiphenyl	33025-41-1	1		1		1										3
2,3',4',5'-Tetrachlorobiphenyl	32598-11-1	1		1		1										3
2,3',4,5'-Tetrachlorobiphenyl	73575-52-7	1		1		1										3
2,4,4',5'-Tetrachlorobiphenyl	32690-93-0	1		1		1										3
2,6,2',6'-Tetrachlorobiphenyl	15968-05-5						1									1
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	1		1		1	1								1	5
3,3',4,5'-Tetrachlorobiphenyl	70362-49-1	1		1		1										3
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3										1	2			1	3
2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8						1		1			1			1	4
2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8	1		1		1	3		2							5
2',3',4',5'-Tetrachloro-3-biphenylol	67651-37-0										1					1
2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	1		1		1	1		1		1	3			1	8
2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5						1		1			1			1	4
2',3',5',6'-Tetrachloro-4,4'-biphenyldiol	100702-98-5											1				1
2,3,7,8,-Tetrachlorodibenzo- <i>p</i> -dioxin	1746-01-6									1						1
Tetrahydrochrysene	104460-72-2					1		1							2	
(<i>rac</i>)-Tetrahydrochrysene							1		1							2
(<i>R,R</i>)-Tetrahydrochrysene							1		1							2
(<i>S,S</i>)-Tetrahydrochrysene							1		1							2
2,2',4,4'-Tetrahydroxybenzil	5394-98-9														1	1
Tetramethylhexestrol	74385-27-6									1	1					2

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERdef	GST-mERdef	GST-rtERdef	hER α	hER α -FP	hER β	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rER β	RUC	Total No. of Assays
Thalidomide	50-35-1														1	1
Toremifene citrate	89778-27-8														1	1
Tosyl Nonylphenol (mixed branched isomers)															1	1
Toxaphene	8001-35-2						1				1	1				3
Triaryl-pyrazole							1		1							2
2,2',5-Trichlorobiphenyl	37680-65-2	1		1		1										3
2,4,6-Trichlorobiphenyl	35693-92-6						1									1
3,3',4-Trichloro-4-biphenylol	124882-64-0										1					1
2',4',6'-Trichloro-4-biphenylol	14962-28-8						2		1		1	3				4
3,4',5-Trichloro-4-biphenylol	4400-06-0											1				1
2,4,5-Trichlorophenoxyacetic acid	93-76-5														1	1
Triethylamine, 2-[p -[6-methoxy-2-phenyl-3-inden-3-yl]phenoxy]hydrochloride	64-96-0												1			1
Triethylene glycol dimethacrylate	109-16-0							1								1
4,2',4'-Trihydroxychalcone	961-29-5														1	1
3,6,4'-Trihydroxyflavone	253195-19-6														1	1
6,7,4'-Trihydroxyisoflavone	17817-31-1														1	1
7,3',4'-Trihydroxyisoflavone	485-63-2														1	1
1,1,2-Triphenylbut-1-ene	63019-13-6														1	1
Triphenylethylene	58-72-0														1	1
Triphenyl phosphate	115-86-6														1	1
Tris-4-(chlorophenyl)methane	27575-78-6									1						1
Tris-4-(chlorophenyl)methanol	30100-80-8									1						1
Vanillin	121-33-5														1	1
Vinclozolin	50471-44-8														2	1
-Zearalanol	26538-44-3														1	1
-Zearalanol	42422-68-4						1							1	1	3
Zearalanone	5975-78-0														1	1
-Zearalenol	36455-72-8	1	1	1	1	1									1	6
-Zearalenol	71030-11-0	1	1	1	1	1									1	6
Zearalenone	17924-92-4	1	1	1	1	1	2	1	2		1				1	10
TOTALS		85	34	99	34	86	133	48	100	66	94	75	44	38	376	

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Appendix F

Median/Single RBA Values for Substances Tested in

Two or More *In Vitro* ER Binding Assays

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Substance	CASRN	RUC		hER α		hER α -FP		hER β		rER β		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
Alachlor	15972-60-8	neg (100)	(1)	neg (50)	(1)										
4-tert -Amylphenol	80-46-6	0.0005	(1)	neg (10)	(1)			neg (10)	(1)						
3 β -Androstenediol	25126-76-5			3	(1)					7	(1)				
5 -Androstane-3 ,17 β -diol	1852-53-5	0.002	(1)												
5 -Androstane-3 ,17 -diol	571-20-0	0.12	(1)	0.07	(1)					0.3	(1)				
5 β -Androstane-3,17-dione	5982-99-0			neg (100)	(1)					neg (100)	(1)				
5 β -Androstenedione	1229-12-5			neg (100)	(1)					neg (100)	(1)				
4-Androstenediol	1156-92-9			0.5	(1)					0.6	(1)				
5-Androstenediol	521-17-5			3.9	(3)			14.1	(2)	17	(1)				
4-Androstenedione	63-05-8			neg (100)	(1)					neg (100)	(1)				
Apigenin	520-36-5	0.028	(1)	0.3	(1)			4	(2)						
Atrazine	1912-24-9	0.0003	(1/2)			neg (2000)	(1)					neg (100)	(1)	neg (100)	(1)
Benz[a]anthracene	56-55-3							neg (10)	(1)						
Benzeneacetoneitrile, -bis(4-hydroxyphenyl)methylene	66422-14-8														
Benzo[a]carbazole	239-01-0							neg (10)	(1)						
Benzo [c]carbazole								neg (10)	(1)						
Benzo[b]fluorene	243-17-4							neg (10)	(1)						
Benzo[b]naphtho[2,1-d]thiophene	239-35-0							neg (10)	(1)						
Benzo[b]naphtho[2,3-d]thiophene	243-46-9							neg (10)	(1)						
Benzo[c]phenanthrene	195-19-7							neg (10)	(1)						
Biochanin A	491-80-5	0.0043	(1)	neg (10)	(1)			neg (10)	(1)						
3,4-Bis(3-hydroxyphenyl)hexane	68266-24-0														
2,2-Bis(p -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	0.754	(3)	0.4	(1)	1.7	(1)	2	(1)			4.80	(1)	4.8	(1)
Bisphenol A	80-05-7	0.056	(5)	0.01	(3)	0.25	(2)	0.0365	(2)	0.33	(1)	0.13	(1)	0.044	(1)
Bisphenol A diglycidyl ether dimethacrylate	1565-94-2	neg (200)	(2)			neg (5000)	(1)								
Bisphenol A dimethacrylate	3253-39-2	0.017	(2)												
Bisphenol C 2	14868-03-2	2.64	(1)												
16 -Bromo-17 β -estradiol	54982-79-5			65	(2)			13.4	(2)						
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl)phenoxy]-	107144-85-4														
1,3-Butanediol, 4-[4-[1,2,3,4-tetrahydro-6-methoxy-2-phenyl 1-naphthenyl)phenoxy]-	107163-56-4														
Butyl benzyl phthalate	85-68-7	0.0017	(2/5)			0.0061	(2)					neg (100)	(1)	neg (100)	(1)
4-tert -Butylphenol	98-54-4	0.0009	(2)	neg (10)	(1)			neg (10)	(1)						
2-Chloro-4-biphenylol	23719-22-4	0.002	(1)												

Substance	CASRN	GST-hER α def	GST-mER α def	GST-rtERdef	MCF-7 cells	MCF-7 cytosol	MUC	RBC
		RBA n	RBA n	RBA n	RBA n	RBA n	RBA n	RBA n
Alachlor	15972-60-8							
4-tert -Amylphenol	80-46-6							
3 β -Androstenediol	25126-76-5							
5 -Androstane-3 ,17 β -diol	1852-53-5					neg (0.1) (1)	neg (10) (1)	
5 -Androstane-3 ,17 -diol	571-20-0					0.005 (1)	0.5 (1)	
5 β -Androstane-3,17-dione	5982-99-0						neg (10) (1)	
5 β -Androstenedione	1229-12-5							
4-Androstenediol	1156-92-9							
5-Androstenediol	521-17-5							1 (1)
4-Androstenedione	63-05-8					0.007 (1)	neg (10) (1)	
Apigenin	520-36-5							
Atrazine	1912-24-9	neg (100) (1)	neg (100) (1)	neg (100) (1)				
Benz[<i>a</i>]anthracene	56-55-3	neg (10) (1)			33 (1)			
Benzeneacetonitrile, -bis(4-hydroxyphenyl)methylene	66422-14-8				8.5 (1)	100 (1)		
Benzo[<i>a</i>]carbazole	239-01-0	neg (10) (1)						
Benzo[<i>c</i>]carbazole		neg (10) (1)						
Benzo[<i>b</i>]fluorene	243-17-4	neg (10) (1)						
Benzo[<i>b</i>]naphtho[2,1-d]thiophene	239-35-0	neg (10) (1)						
Benzo[<i>b</i>]naphtho[2,3-d]thiophene	243-46-9	neg (10) (1)						
Benzo[<i>c</i>]phenanthrene	195-19-7	neg (10) (1)						
Biochanin A	491-80-5							
3,4-Bis(3-hydroxyphenyl)hexane	68266-24-0				20 (1)	10 (1)		
2,2-Bis(<i>p</i> -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	1.2 (1)	1.2 (1)	14 (1)			1.2 (1)	
Bisphenol A	80-05-7	0.008 (1)	0.0086 (1)	0.21 (1)	0.006 (1)	1 (1)	0.0115 (1)	0.0013 (1)
Bisphenol A diglycidyl ether dimethacrylate	1565-94-2							
Bisphenol A dimethacrylate	3253-39-2							0.00047 (1)
Bisphenol C 2	14868-03-2				0.3 (1)	2 (1)		
16 -Bromo-17 β -estradiol	54982-79-5							
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl)phenoxy]-	107144-85-4				7 (1)	30 (1)		
1,3-Butanediol, 4-[4-[1,2,3,4-tetrahydro-6-methoxy-2-phenyl 1-naphthenyl)phenoxy]-	107163-56-4				0.06 (1)	0.1 (1)		
Butyl benzyl phthalate	85-68-7	neg (100) (1)	neg (100) (1)	neg (100) (1)				neg (100) (1)
4-tert -Butylphenol	98-54-4							
2-Chloro-4-biphenylol	23719-22-4						0.04 (1)	

Median/Single RBA Values for Substances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	RUC		hER α		hER α -FP		hER β		rER β		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
4-Chloro-4'-biphenylol	28034-99-3	0.007	(1)												
11 -Chloromethylestradiol	71794-60-0														
Chrysene	218-01-9	neg (10)	(1)					neg (10)	(1)						
Chrysin	480-40-0	neg (100)	(1)	neg (10)	(1)			neg (10)	(1)						
<i>trans</i> -Clomiphene	911-45-5			25	(1)					12	(1)				
Corticosterone	50-22-6	neg (100)	(1)	neg (100)	(1)					neg (100)	(1)				
Coumestrol	479-13-0	1.86	(2)	34	(3)	12	(1)	120	(2)	185	(1)	3.1	(1)	0.7	(1)
Cyclofenil diphenol	5189-40-2														
Daidzein	486-66-8	0.023	(1)	0.1	(3)	0.2	(1)	1	(3)						
<i>o,p'</i> -DDD	53-19-0	0.009	(1/2)	neg (50)	(2)			neg (10)	(1)						
<i>p,p'</i> -DDD	72-54-8	0.0003	(1/3)	0.009	(1/2)			neg (10)	(1)						
<i>o,p'</i> -DDE	3424-82-6	0.0009	(1/2)	neg (10)	(1)			neg (10)	(1)			neg (100)	(1)	neg (100)	(1)
<i>p,p'</i> -DDE	72-55-9	0.0003	(1/3)	neg (10)	(1)			neg (10)	(1)			neg (100)	(1)	neg (100)	(1)
<i>o,p'</i> -DDT	789-02-6	0.013	(5)	0.055	(2)	0.4	(1)	0.02	(1)			neg (100)	(1)	0.086	(1)
(-)- <i>o,p'</i> -DDT	58633-26-4	0.029	(1)												
(+)- <i>o,p'</i> -DDT	58633-27-5	neg (20)	(1)												
<i>p,p'</i> -DDT	50-29-3	0.0003	(1/3)	neg (10)	(1)	0.041	(1)	neg (10)	(1)			neg (100)	(1)	neg (100)	(1)
Dehydroepiandrosterone	53-43-0			0.04	(1)					0.07	(1)	neg (100)	(1)	neg (100)	(1)
3-Deoxyestradiol	2529-64-8	0.5	(1)												
3-Deoxyestradiol	2529-64-8														
17-Desoxyestradiol	53-63-4	18.16	(3)												
17-Desoxyestradiol	53-63-4	18.2	(3)												
Dibutyl phthalate	84-74-2	0.0027	(1)			neg (5000)	(1)								
Dibutyl benzyl phthalate												neg (100)	(1)	neg (100)	(1)
2',5'-Dichloro-4-biphenylol	53905-28-5	0.036	(1)	0.033	(1)										
Dieldrin	60-57-1	neg (100)	(2)	neg (10)	(1)	0.0005	(1)								
Dienestrol	84-17-3	37.5	(1)	223	(1)					404	(1)				
Diethylhexyl phthalate	117-81-7	neg (1000)	(2)			neg (5000)	(1)								
Diethylstilbestrol	56-53-1	200	(11)	236	(3)	124	(4)	160.5	(2)	295	(1)	107	(1)	130	(1)
3,3'-Diethylstilbestrol	5959-71-7														
(<i>rac</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				14	(1)			67	(1)						
5,11- <i>trans</i> -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				221	(1)			432	(1)						
(5 <i>R</i> ,11 <i>R</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				23	(1)			144	(1)						
(5 <i>S</i> ,11 <i>S</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				0.9	(1)			14	(1)						
Dihydrogenistein				0.143	(1)			18.5	(1)						
5 -Dihydrotestosterone	521-18-6	0.0135	(2)	0.05	(1)	0.0095	(1)			0.17	(1)	0.38	(1)	0.0085	(1)

Substance	CASRN	GST-hER α def	GST-mER α def	GST-rtERdef	MCF-7 cells	MCF-7 cytosol	MUC	RBC
		RBA n	RBA n	RBA n	RBA n	RBA n	RBA n	RBA n
4-Chloro-4'-biphenylol	28034-99-3						0.0365 (2)	
11 -Chloromethylestradiol	71794-60-0				96 (1)	100 (1)		
Chrysene	218-01-9	neg (10) (1)			13 (1)			
Chrysin	480-40-0							
<i>trans</i> -Clomiphene	911-45-5							0.0081 (1)
Corticosterone	50-22-6							
Coumestrol	479-13-0	0.81 (1)	0.33 (1)	0.24 (1)	12 (1)	13 (1)		
Cyclofenil diphenol	5189-40-2				0.5 (1)	5 (1)		
Daidzein	486-66-8							
<i>o,p'</i> -DDD	53-19-0							
<i>p,p'</i> -DDD	72-54-8							
<i>o,p'</i> -DDE	3424-82-6	neg (100) (1)	neg (100) (1)	0.11 (1)				
<i>p,p'</i> -DDE	72-55-9	neg (100) (1)	neg (100) (1)	0.042 (1)				neg (100) (1)
<i>o,p'</i> -DDT	789-02-6	neg (100) (1)	0.0073 (1)	0.43 (1)		0.0003 (1)	0.21 (1)	0.00059 (1)
(-)- <i>o,p'</i> -DDT	58633-26-4				0.013 (1)			
(+)- <i>o,p'</i> -DDT	58633-27-5				0.0001 (1)			
<i>p,p'</i> -DDT	50-29-3	neg (100) (1)	neg (100) (1)	0.165 (1)				
Dehydroepiandrosterone	53-43-0	neg (100) (1)	neg (100) (1)	0.028 (1)				
3-Deoxyestradiol	2529-64-8				0.6 (1)	8 (1)		
3-Deoxyestradiol	2529-64-8				0.6 (1)	8 (1)		
17-Desoxyestradiol	53-63-4				0.5 (1)	40 (1)		
17-Desoxyestradiol	53-63-4				0.5 (1)	40 (1)		
Dibutyl phthalate	84-74-2							neg (100) (1)
Dibutyl benzyl phthalate		neg (100) (1)	neg (100) (1)	0.2 (1)				
2',5'-Dichloro-4-biphenylol	53905-28-5						0.459 (2)	
Dieldrin	60-57-1						neg (10) (1)	
Dienestrol	84-17-3							
Diethylhexyl phthalate	117-81-7							
Diethylstilbestrol	56-53-1	91 (1)	84 (1)	165 (1)	84 (1)	100 (2)	113 (7)	1551.5 (2)
3,3'-Diethylstilbestrol	5959-71-7				17.5 (1)	3 (1)		
(<i>rac</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
5,11- <i>trans</i> -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
(5 <i>R</i> ,11 <i>R</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
(5 <i>S</i> ,11 <i>S</i>)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
Dihydrogenistein								
5 -Dihydrotestosterone	521-18-6	0.049 (1)	0.04 (1)	0.034 (1)			neg (10) (1)	

Substance	CASRN	RUC		hER α		hER α -FP		hER β		rER β		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
4,4'-Dihydroxybiphenyl	92-88-6			neg (10)	(1)			0.03	(1)						
, -Dimethylstilbestrol	552-80-7	14.5	(1)												
5,11- <i>trans</i> -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				222	(1)			254	(1)						
(5 <i>R</i> , 11 <i>R</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				24	(1)			76	(1)						
(5 <i>S</i> , 11 <i>S</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				9.3	(1)			75	(1)						
Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9														
Phenol, 4-(1 <i>Z</i>) 1-2-diphenyl-1-butenyl)-	69967-80-2														
5,11- <i>trans</i> -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol				33.6	(1)			92.3	(1)						
(5 <i>R</i> , 11 <i>R</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol				5.2	(1)			26	(1)						
(5 <i>S</i> , 11 <i>S</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol				1.6	(1)			5.1	(1)						
Droloxifene	82413-20-5	15.2	(1)												
, -Endosulfan	115-29-7	0.00044	(1/2)	neg (10)	(1)			neg (10)	(1)						
-Endosulfan	959-98-8			neg (10)	(1)							neg (100)	(1)	neg (100)	(1)
-Endosulfan	33213-65-9			neg (10)	(1)							neg (100)	(1)	neg (100)	(1)
17-Epiestriol	1228-72-4			29.0	(1)			80	(1)						
Equol	531-95-3	0.15	(1)	0.33	(1)			58.8	(1)						
16 -Estradiol	1090-04-6														
17 -Estradiol	57-91-0	26.5	(2)	32.5	(2)			2	(1)	11	(1)				
Estradiol 3-benzoate	50-50-0											13	(1)	15	(1)
17 -Estradiol 3-methyl ether	1035-77-4	0.7	(1)												
9-Estratetraene-3,17 -diol	791-69-5														
Estra-1,3,5(10)-triene-3,17 -diol, 14,15 -epoxy-	79581-12-7														
Estra-1,3,5(10)-triene-3,17 -diol, 14 ,15 -epoxy-	79645-49-1														
Estra-1,3,5,(10),trien-3,14,17 -triol	16288-09-8														
Estriol	50-27-1	14.4	(2)	14	(1)					21	(1)	30	(1)	11	(1)
Estrone	53-16-7	48	(4)	60	(1)	2.1	(1)			37	(1)	60	(1)	50	(1)
Estrone 3-sulfate	481-97-0			neg (100)	(1)					neg (100)	(1)				
17 -Ethinyl estradiol	57-63-6	173	(4)									139	(1)	171	(1)
Flavone	525-82-6	neg (100)	(1)	neg (10)	(1)			neg (10)	(1)						
Formononetin	485-72-3	0.0013	(1)	neg (10)	(1)			neg (10)	(1)						
Genistein	446-72-0	0.56	(2)	2.36	(4)	1.6	(1)	13	(3)	36	(1)	1.3	(1)	0.78	(1)
Genistin	529-59-9	neg (100)	(1)					0.014	(1)						
Glycitein	40957-83-3			0.016	(1)			0.91	(1)						
2,2',3,3',4',5,6-Heptachlorobiphenyl	52663-70-4											neg (10)	(1)		
2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1											neg (10)	(1)		

Substance	CASRN	GST-hER α def	GST-mER α def	GST-rtERdef	MCF-7 cells	MCF-7 cytosol	MUC	RBC
		RBA n	RBA n	RBA n	RBA n	RBA n	RBA n	RBA n
4,4'-Dihydroxybiphenyl	92-88-6						neg (5) (1)	
, -Dimethylstilbestrol	552-80-7							129 (1)
5,11- <i>trans</i> -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
(5 <i>R</i> ,11 <i>R</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
(5 <i>S</i> ,11 <i>S</i>)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9				2 (1)	15 (1)		
Phenol, 4-(1 <i>Z</i>) 1-2-diphenyl-1-butenyl)-	69967-80-2				0.4 (1)	1 (1)		
5,11- <i>trans</i> -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
(5 <i>R</i> ,11 <i>R</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
(5 <i>S</i> ,11 <i>S</i>)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol								
Droloxifene	82413-20-5				0.2 (1)	2.5 (1)		
, -Endosulfan	115-29-7						neg (5) (1)	neg (100) (1)
-Endosulfan	959-98-8	neg (100) (1)	neg (100) (1)	0.012 (1)				
-Endosulfan	33213-65-9	neg (100) (1)	neg (100) (1)	neg (100) (1)		0.00024 (1)		
17-Epiestriol	1228-72-4							
Equol	531-95-3							
16 -Estradiol	1090-04-6					17.9 (2)		66 (1)
17 -Estradiol	57-91-0					0.22 (1)	1000 (1)	49 (1)
Estradiol 3-benzoate	50-50-0	10 (1)	12 (1)	9 (1)				
17 -Estradiol 3-methyl ether	1035-77-4							3 (1)
9-Estratetraene-3,17 -diol	791-69-5				37 (1)	80 (1)		
Estra-1,3,5(10)-triene-3,17 -diol, 14,15 -epoxy-	79581-12-7				10 (1)	5 (1)		
Estra-1,3,5(10)-triene-3,17 -diol, 14 ,15 -epoxy-	79645-49-1				0.08 (1)	neg (0.1) (1)		
Estra-1,3,5,(10),trien-3,14,17 -triol	16288-09-8				1.5 (1)	2 (1)		
Estriol	50-27-1	28 (1)	13 (1)	3.7 (1)	20 (1)	13 (3)	100 (1)	16 (1)
Estrone	53-16-7	45 (1)	28 (1)	14 (1)	19 (1)	13 (3)	100 (1)	66 (1)
Estrone 3-sulfate	481-97-0							
17 -Ethinyl estradiol	57-63-6	127 (1)	118 (1)	108 (1)				191 (1)
Flavone	525-82-6							
Formononetin	485-72-3							
Genistein	446-72-0	0.46 (1)	0.33 (1)	0.44 (1)		2.31 (2)		
Genistin	529-59-9							
Glycitein	40957-83-3							
2,2',3,3',4',5,6-Heptachlorobiphenyl	52663-70-4	neg (10) (1)		0.031 (1)				
2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1	neg (10) (1)		0.031 (1)				

Substance	CASRN	RUC	hER α	hER α -FP	hER β	rER β	GST-aERdef	GST-cERdef
		RBA n	RBA n	RBA n	RBA n	RBA n	RBA n	RBA n
2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6						neg (10) (1)	
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1						neg (10) (1)	
2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3						0.025 (1)	
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0						neg (10) (1)	
2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7						0.025 (1)	
2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7						neg (10) (1)	
2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8						neg (10) (1)	
2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3		0.1 (1)		0.1 (1)		neg (10) (1)	
2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8		0.09 (2)		0.1 (2)		neg (10) (1)	
2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7		0.1 (1)		0.1 (1)		neg (10) (1)	
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3						neg (10) (1)	
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2						neg (10) (1)	
2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0						neg (10) (1)	
2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0						neg (10) (1)	
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5						neg (10) (1)	
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1						neg (10) (1)	
2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7						neg (10) (1)	
2,3',4,4',5',6-Hexachlorobiphenyl	59291-65-5						neg (10) (1)	
2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2		neg (50) (1)					
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6						neg (10) (1)	
2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1		0.07 (1)		0.06 (1)		neg (10) (1)	
2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7		0.03 (1)		0.04 (1)		neg (10) (1)	
Hexestrol	84-16-2	299.8 (2)	302 (1)			234 (1)		
3-Hydroxybenzo[b]naphtho[2,1-d]thiophene					2.5 (1)			
2-Hydroxybenzo[c]phenanthrene					3.1 (1)			
3-Hydroxybenzo[b]phenanthro[2,3-d]thiophene					5 (1)			
2-Hydroxychrysene	65945-06-4				13.33 (1)			
11 -Hydroxyestradiol	5444-22-4	7 (1)						
14 -Hydroxyestradiol	60183-66-6							
2-Hydroxyestradiol	362-05-0		7 (1)			11 (1)		
4-Hydroxyestradiol	5976-61-4		13 (1)			7 (1)		
2-Hydroxyestrone	362-06-1		2 (1)		0.2 (1)			
2-Hydroxy-5-methylchrysene					19.3 (1)			
8-Hydroxy-5-methylchrysene					3.1 (1)			
4-Hydroxytamoxifen	68047-06-3	175.2 (1)	178 (3)	21.5 (3)	147 (2)	339 (1)	243 (1)	168 (1)
ICI 164384	98007-99-9	14.5 (1)	121.5 (2)		93 (1)	166 (1)	28 (1)	62 (1)
ICI 182780	129453-61-8	235.2 (2)	32 (1)		25 (1)			

Substance	CASRN	GST-hER α def		GST-mER α def		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3	0.024	(1)			0.78	(1)								
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7	0.024	(1)			0.24	(1)								
2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7	neg (10)	(1)			neg (10)	(1)								
2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8	neg (10)	(1)			neg (10)	(1)								
2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	neg (10)	(1)			neg (10)	(1)								
2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7	neg (10)	(1)			neg (10)	(1)								
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0	neg (10)	(1)			0.031	(1)								
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5	neg (10)	(1)			neg (10)	(1)								
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	neg (10)	(1)			neg (10)	(1)								
2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7	neg (10)	(1)			neg (10)	(1)								
2,3',4,4',5',6-Hexachlorobiphenyl	59291-65-5	neg (10)	(1)			neg (10)	(1)								
2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2											0.27	(1)		
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	neg (10)	(1)			neg (10)	(1)								
2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	neg (10)	(1)			neg (10)	(1)								
Hexestrol	84-16-2							58	(1)	100	(1)			74	(1)
3-Hydroxybenzo[b]naphtho[2,1-d]thiophene		1.83	(1)												
2-Hydroxybenzo[c]phenanthrene		2.2	(1)												
3-Hydroxybenzo[b]phenanthro[2,3-d]thiophene		2.4	(1)												
2-Hydroxychrysene	65945-06-4	5.8	(1)												
11-Hydroxyestradiol	5444-22-4							4	(1)	8.9	(2)			4	(1)
14-Hydroxyestradiol	60183-66-6							3.5	(1)	10	(1)				
2-Hydroxyestradiol	362-05-0														
4-Hydroxyestradiol	5976-61-4														
2-Hydroxyestrone	362-06-1														
2-Hydroxy-5-methylchrysene		19.6	(1)												
8-Hydroxy-5-methylchrysene		3.1	(1)												
4-Hydroxytamoxifen	68047-06-3	155	(1)	212	(1)	272	(1)	2.9	(1)	100	(1)	14.4	(1)		
ICI 164384	98007-99-9	42	(1)	45	(1)	327	(1)								
ICI 182780	129453-61-8													500	(1)

Substance	CASRN	RUC		hER α		hER α -FP		hER β		rER β		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
(E)-17 β -Iodovinylestradiol	82123-96-4	7	(1)												
(Z)-17 β -Iodovinylestradiol	177159-09-0	63	(1)												
Ipriflavone	35212-22-7			neg (10)	(1)			neg (10)	(1)						
Kaempferol	520-18-3	0.025	(1)	0.1	(1)			2.5	(2)						
Kepone	143-50-0	0.03	(3)	0.06	(1)	0.2	(1)	0.1	(1)			0.011	(1)	0.011	(1)
16-Ketoestradiol	566-75-6			1.3	(1)			0.9	(1)						
6-Ketoestradiol	571-92-6														
Mestranol	72-33-3	1.26	(2)			0.0096	(1)								
Methoxychlor (<i>p,p'</i> -)	72-43-5	0.001	(3/5)	0.01	(1/2)			neg (10)	(1)	0.13	(1)	neg (100)	(1)	neg (100)	(1)
(E)-11 β -methoxy-17 β -iodovinylestradiol	90857-55-9	11	(1)												
(Z)-11 β -Methoxy-17 β -iodovinylestradiol	177159-11-4	41	(1)												
11 β -Methylestradiol-17	23637-93-6	124	(1)												
Moxestrol	34816-55-2	13.8	(1)	43	(1)					5	(1)				
Nafoxidine	1845-11-0	0.72	(1)	44	(1)					16	(1)				
Naringenin	480-41-1	0.0075	(1)	0.01	(1)			0.155	(2)			0.065	(1)	0.0082	(1)
4- <i>n</i> -Nonylphenol	25154-52-3			0.0255	(2)			0.0765	(2)						
<i>p</i> -Nonylphenol	104-40-5	0.033	(10)			0.4	(2)								
Norethindrone	68-22-4			0.07	(1)					0.01	(1)				
Norethynodrel	68-23-5	0.22	(2)	0.7	(1)					0.22	(1)				
19-Nortestosterone	434-22-0			0.01	(1)					0.23	(1)				
2,2',3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7											neg (10)	(1)		
4-Octylphenol	1806-26-4	0.005	(1)	0.02	(1)			0.07	(1)						
4- <i>tert</i> -Octylphenol	140-66-9	0.197	(3)	0.01	(1)	0.2	(1)	0.03	(1)			0.099	(1)	0.57	(1)
2,2',3,3',6-Pentachlorobiphenyl	52663-60-2											neg (10)	(1)		
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8											neg (10)	(1)		
2,2',3,4',6-Pentachlorobiphenyl	68194-05-8											neg (10)	(1)		
2,2',3,5',6-Pentachlorobiphenyl	38379-99-6											neg (10)	(1)		
2,2',4,4',5-Pentachlorobiphenyl	38380-01-7											neg (10)	(1)		
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2											neg (10)	(1)		
2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8											0.025	(1)		
2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9											neg (10)	(1)		
2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1											neg (10)	(1)		
3,3',4,4',5,-Pentachlorobiphenyl	57465-28-8											neg (10)	(1)		
2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	0.082	(1)	0.11	(1)			0.11	(1)						
2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4			0.03	(1)			0.11	(1)			neg (10)	(1)		
2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	0.041	(1)	0.13	(1)			0.12	(1)						
2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	0.036	(1)	0.1	(1)			0.13	(1)						
2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	0.12	(1)	0.3	(1)			0.2	(1)						

Substance	CASRN	GST-hER α def	GST-mER α def	GST-rtERdef	MCF-7 cells	MCF-7 cytosol	MUC	RBC
		RBA n	RBA n	RBA n	RBA n	RBA n	RBA n	RBA n
(E)-17 -Iodovinylestradiol	82123-96-4					17 (1)		
(Z)-17 -Iodovinylestradiol	177159-09-0					51 (1)		
Ipriflavone	35212-22-7							
Kaempferol	520-18-3							
Kepone	143-50-0	0.0069 (1)	0.0035 (1)	0.054 (1)			neg (5) (1)	
16-Ketoestradiol	566-75-6					0.9 (1)		14 (1)
6-Ketoestradiol	571-92-6				15 (1)	20 (1)		
Mestranol	72-33-3							8 (1)
Methoxychlor (<i>p,p'</i> -)	72-43-5	neg (100) (1)	neg (100) (1)	0.95 (1)		neg (1000) (1)	neg (5) (1)	0.0031 (1)
(E)-11 -methoxy-17 -iodovinylestradiol	90857-55-9					17 (1)		
(Z)-11 -Methoxy-17 -iodovinylestradiol	177159-11-4					31 (1)		
11 -Methylestradiol-17	23637-93-6				100 (1)	100 (1)		
Moxestrol	34816-55-2						4.25 (1)	
Nafoxidine	1845-11-0				0.1 (1)	5 (1)		
Naringenin	480-41-1	neg (100) (1)	neg (100) (1)	0.039 (1)				
4- <i>n</i> -Nonylphenol	25154-52-3						neg (5) (1)	0.0011 (1)
<i>p</i> -Nonylphenol	104-40-5				0.026 (1)	0.021 (1)		
Norethindrone	68-22-4							
Norethynodrel	68-23-5							
19-Nortestosterone	434-22-0						0.1 (1)	
2,2',3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7	neg (10) (1)		neg (10) (1)				
4-Octylphenol	1806-26-4				0.072 (1)	1 (1)		neg (100) (1)
4- <i>tert</i> -Octylphenol	140-66-9	0.12 (1)	0.17 (1)	3.2 (1)				
2,2',3,3',6-Pentachlorobiphenyl	52663-60-2	neg (10) (1)		neg (10) (1)				
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	neg (10) (1)		neg (10) (1)				
2,2',3,4',6-Pentachlorobiphenyl	68194-05-8	neg (10) (1)		0.031 (1)				
2,2',3,5',6-Pentachlorobiphenyl	38379-99-6	neg (10) (1)		neg (10) (1)				
2,2',4,4',5-Pentachlorobiphenyl	38380-01-7	neg (10) (1)		neg (10) (1)				
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	neg (10) (1)		neg (10) (1)				
2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8	0.024 (1)		0.24 (1)			0.88 (1)	
2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9	neg (10) (1)		neg (10) (1)				
2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1	neg (10) (1)		0.031 (1)				
3,3',4,4',5,-Pentachlorobiphenyl	57465-28-8	neg (10) (1)		neg (10) (1)				
2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9						0.072 (1)	
2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4	neg (10) (1)		neg (10) (1)		1 (1)		
2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6						neg (1000) (1)	
2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4						0.033 (1)	
2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2						0.044 (1)	

Substance	CASRN	RUC		hER α		hER α -FP		hER β		rER β		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	0.14	(1)	0.09	(1)			0.03	(1)						
2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3			0.01	(1)			neg (10)	(1)						
2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	0.068	(1)	0.06	(1)			0.04	(1)						
Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis	107144-81-0														
Phenol, 4-[7-[2 dimethylamino)ethoxy]-11-ethyl]dibenzo[<i>b,f</i>]thiepin-10-yl]	85850-74-4	63	(1)												
Phenol, 4-[3-[2 dimethylamino)ethoxy]-11-ethyl]dibenzo[<i>b,f</i>]thioctin-12-yl]	85850-81-3	52	(1)												
Phenol, 4-[2-[2 dimethylamino)ethoxy]-6-ethyl-11,12-dihydrodibenzo[<i>a,e</i>]cycloocten-5-yl]	85870-75-5	50	(1)												
Phenol, 3-[2-dimethylamino-ethoxy]-10-ethyl-4-hydroxy-phenyl dibenzo-[<i>b,f</i>]oxepin	85850-80-2	6.1	(1)												
Phenol, 4-[1-[4-[2-(dimethylamino)ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (<i>E</i>)	96474-35-0														
Phenol, 4-[2-nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy] phenyl]-ethenyl]	107144-84-3														
Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-	91221-46-4														
4-Phenylphenol	92-69-3	0.001	(1)												
Phloretin	60-82-2	0.069	(1)	0.2	(1)			0.7	(1)						
Progesterone	57-83-0	0.0003	(1/3)	neg (100)	(2)			neg (10)	(1)	neg (100)	(1)				
Promegestone	34184-77-5	0.22	(1/2)												
Propylpyrazoletriol				49	(1)			0.12	(1)						
Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (<i>E</i>) (9 CI)	77413-87-7														
Quercetin	117-39-5	neg (100)	(1)	0.0100	(1)			0.04	(1)			0.016	(1)	0.0039	(1)
6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1														
7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2														
Raloxifene	84449-90-1			60.4	(2)			21	(2)						
Simazine	122-34-9	neg (33.3)	(1)			neg (2000)	(1)					neg (100)	(1)	neg (100)	(1)
β -Sitosterol	83-46-5	neg (1000)	(1)	neg (100)	(1)					neg (100)	(1)	neg (100)	(1)	neg (100)	(1)
Tamoxifen	10540-29-1	3.1	(4)	4	(3)	3.03	(2)	2.34	(2)	6	(1)	10	(1)	16	(1)
Testosterone	58-22-0	0.01	(1/2)	neg (100)	(2)			neg (10)	(1)	neg (100)	(1)				
2,2',3,3'-Tetrachlorobiphenyl	3844-93-8											neg (10)	(1)		
2,2',3,4'-Tetrachlorobiphenyl	52663-59-9											neg (10)	(1)		
2,2',3,6'-Tetrachlorobiphenyl	41464-47-5											neg (10)	(1)		
2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	neg (100)	(1)									neg (10)	(1)		
2,2',4,5'-Tetrachlorobiphenyl	41464-40-8											neg (10)	(1)		
2,2',4,6'-Tetrachlorobiphenyl	68194-04-7											neg (10)	(1)		
2,2',6,6'-Tetrachlorobiphenyl	15968-05-5			neg (100)	(1)			neg (100)	(1)			neg (10)	(1)		
2,3,3',5'-Tetrachlorobiphenyl	41464-49-7											neg (10)	(1)		

Substance	CASRN	GST-hER α def	GST-mER α def	GST-rtERdef	MCF-7 cells	MCF-7 cytosol	MUC	RBC
		RBA n	RBA n	RBA n	RBA n	RBA n	RBA n	RBA n
2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3						0.013 (1)	
2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3			neg (10) (1)		0.1 (2)	0.072 (1)	
2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7						0.031 (1)	
Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis	107144-81-0				26 (1)	20 (1)		
Phenol, 4-[7-[2 dimethylamino]ethoxy]-11-ethyl-dibenzo[<i>b,f</i>]thiepin-10-yl]	85850-74-4				0.3 (1)	63 (1)		
Phenol, 4-[3-[2 dimethylamino]ethoxy]-11-ethyl-dibenzo[<i>b,f</i>]thioctin-12-yl]	85850-81-3				2.5 (1)	50 (1)		
Phenol, 4-[2-[2 dimethylamino]ethoxy]-6-ethyl-11,12-dihydrodibenzo[<i>a,e</i>]cycloocten-5-yl]	85870-75-5				1.3 (1)	50 (1)		
Phenol, 3-[2-dimethylamino-ethoxy]-10-ethyl-4-hydroxy-phenyl dibenzo-[<i>b,f</i>]oxepin	85850-80-2				0.1 (1)	6 (1)		
Phenol, 4-[1-[4-[2-(dimethylamino)ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (<i>E</i>)	96474-35-0				0.4 (1)	100 (1)		
Phenol, 4-[2-nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy] phenyl]-ethenyl]	107144-84-3				2.1 (1)	100 (1)		
Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-	91221-46-4				2 (1)	100 (1)		
4-Phenylphenol	92-69-3						neg (5) (1)	
Phloretin	60-82-2							
Progesterone	57-83-0						neg (10) (1)	
Promegestone	34184-77-5						neg (10) (1)	
Propylpyrazoneletriol								
Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (<i>E</i>) (9 CI)	77413-87-7				0.07 (1)	11 (1)		
Quercetin	117-39-5	neg (100) (1)	neg (100) (1)	0.042 (1)		neg (25) (1)		
6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1				22 (1)	33 (1)		
7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2				3 (1)	9 (1)		
Raloxifene	84449-90-1							
Simazine	122-34-9	neg (100) (1)	neg (100) (1)	neg (100) (1)				
β -Sitosterol	83-46-5	neg (100) (1)	neg (100) (1)	neg (100) (1)				
Tamoxifen	10540-29-1	11 (1)	10 (1)	25 (1)	0.06 (1)	1 (1)	6.55 (1)	0.017 (1)
Testosterone	58-22-0						neg (10) (1)	neg (100) (1)
2,2',3,3'-Tetrachlorobiphenyl	3844-93-8	neg (10) (1)		neg (10) (1)				
2,2',3,4'-Tetrachlorobiphenyl	52663-59-9	neg (10) (1)		0.024 (1)				
2,2',3,6'-Tetrachlorobiphenyl	41464-47-5	neg (10) (1)		0.031 (1)				
2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	neg (10) (1)		0.031 (1)				
2,2',4,5'-Tetrachlorobiphenyl	41464-40-8	neg (10) (1)		neg (10) (1)				
2,2',4,6'-Tetrachlorobiphenyl	68194-04-7	neg (10) (1)		0.0310 (1)				
2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	neg (10) (1)		0.0310 (1)				
2,3,3',5'-Tetrachlorobiphenyl	41464-49-7	neg (10) (1)		neg (10) (1)				

Substance	CASRN	RUC	hER α	hER α -FP	hER β	rER β	GST-aERdef	GST-cERdef
		RBA n	RBA n	RBA n	RBA n	RBA n	RBA n	RBA n
2,3,4,4'-Tetrachlorobiphenyl	33025-41-1						neg (10) (1)	
2,3',4',5-Tetrachlorobiphenyl	32598-11-1						neg (10) (1)	
2,3',4,5'-Tetrachlorobiphenyl	73575-52-7						neg (10) (1)	
2,4,4',5-Tetrachlorobiphenyl	32690-93-0						neg (10) (1)	
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	neg (300) (1)	neg (50) (1)				neg (10) (1)	
3,3',4,5-Tetrachlorobiphenyl	70362-49-1						neg (10) (1)	
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	0.001 (1)						
2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	0.0005 (1)	0.3 (1)		0.5 (1)			
2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.3 (3)		0.25 (1)		0.5 (1)	
2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	0.23 (1)	3.4 (1)		7.2 (1)		0.5 (1)	
2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	neg (1000) (1)	0.18 (1)		0.23 (1)			
Tetrahydrochrysene	104460-72-2		3 (1)		6.5 (1)			
(rac) -Tetrahydrochrysene			2.5 (1)		25 (1)			
(R,R) -Tetrahydrochrysene			3.6 (1)		25 (1)			
(S,S) -Tetrahydrochrysene			0.83 (1)		1.3 (1)			
Tetramethylhexestrol	74385-27-6							
Toxaphene	8001-35-2		neg (10) (1)					
triaryl-Pyrazole			60 (1)		18 (1)			
2,2',5-Trichlorobiphenyl	37680-65-2						neg (10) (1)	
2,4,6-Trichloro-4'-biphenylol	35693-92-6		0.747 (1)					
2',4',6'-Trichloro-4-biphenylol	14962-28-8		1.58 (2)		4.7 (1)			
-Zearalanol	42422-68-4	0.64 (1)	16 (1)			14 (1)		
-Zearalenol	36455-72-8	43 (1)					36 (1)	70 (1)
-Zearalenol	71030-11-0	0.2 (1)					4.2 (1)	23 (1)
Zearalenone	17924-92-4	44.1 (1)	8.5 (2)	22 (1)	11.5 (2)		12 (1)	33 (1)

RBA data presented as median RBA value (in micromolar) for two or more experiments using the same assay, or as a single value if only one experiment had been conducted. Neg = negative for ER binding; number in parenthesis indicates the highest dose tested.

n = the number of experiments conducted. If two numbers are in parenthesis, the first number indicates the number of positive experiments, while the second number indicates the number of experiments conducted.

Substance	CASRN	GST-hER α def		GST-mER α def		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
2,3,4,4'-Tetrachlorobiphenyl	33025-41-1	neg (10)	(1)			neg (10)	(1)								
2,3',4',5-Tetrachlorobiphenyl	32598-11-1	neg (10)	(1)			neg (10)	(1)								
2,3',4,5'-Tetrachlorobiphenyl	73575-52-7	neg (10)	(1)			neg (10)	(1)								
2,4,4',5-Tetrachlorobiphenyl	32690-93-0	neg (10)	(1)			neg (10)	(1)								
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	neg (10)	(1)			neg (10)	(1)								
3,3',4,5-Tetrachlorobiphenyl	70362-49-1	neg (10)	(1)			neg (10)	(1)								
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3									0.4	(1)	0.102	(2)		
2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8											0.018	(1)		
2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8	0.48	(1)			1.03	(1)								
2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	2.4	(1)			1.15	(1)			3.2	(1)	1.11	(3)		
2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5											neg (1000)	(1)		
Tetrahydrochrysene	104460-72-2														
(rac) -Tetrahydrochrysene															
(R,R) -Tetrahydrochrysene															
(S,S) -Tetrahydrochrysene															
Tetramethylhexestrol	74385-27-6							2	(1)	1.5	(1)				
Toxaphene	8001-35-2									0.0003	(1)	neg (10)	(1)		
triaryl-Pyrazole															
2,2',5-Trichlorobiphenyl	37680-65-2	neg (10)	(1)			neg (10)	(1)								
2,4,6-Trichloro-4'-biphenylol	35693-92-6											5.37	(1)		
2',4',6'-Trichloro-4-biphenylol	14962-28-8									6.3	(1)	2.38	(3)		
-Zearalanol	42422-68-4														
-Zearalenol	36455-72-8	48	(1)	53	(1)	267	(1)								
-Zearalenol	71030-11-0	13	(1)	11	(1)	91	(1)								
Zearalenone	17924-92-4	9.3	(1)	12	(1)	82	(1)			18	(1)				

RBA data presented as median RBA value (in micromolar) for two or more experiments using the same assay, or as a single value if only one experiment had been conducted. Neg = negative for ER binding; number in parenthesis indicates the highest dose tested.

n = the number of experiments conducted. If two numbers are in parenthesis, the first number indicates the number of positive experiments, while the second number indicates the number of experiments conducted.